IXTOC I: CHEMICAL CHARACTERIZATION AND ACUTE BIOLOGICAL EFFECTS

P. L. Parker (Editor)

Final Report

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SECTION A

CHEMICAL CHARACTERIZATION OF IXTOC I SAMPLES AND OIL-SEAWATER MIXTURES USED FOR TOXICITY STUDIES

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INTRODUCTION

The chemical component of these studies had two major objectives: 1) to characterize the composition of various IXTOC I oil samples representative of different stages of weathering and 2) to characterize oil-seawater mixtures prepared from the specific mousse sample tested for acute toxicity.

MATERIALS AND METHODS

Samples of IXTOC I oil, mousse and beach tar were obtained from several government and private sources. About 25 ℓ of mousse were collected by personnel aboard the U. S. Coast Guard ship Pt. Baker at 22°50'N and 96°26'W. The Pt. Baker mousse was used for all biological toxicity studies. Five samples of oil collected from a beach in Mexico were also obtained from the Coast Guard. A sample of one of the first IXTOC I tarballs to come ashore on South Padre Island, Texas was supplied by Mr. Craig Hooper of NOAA. A sample of mousse collected near the well site 40 hrs after the blowout was received from Dr. Alfonso Botello of Mexico. Another sample of "fresh" mousse was obtained from PEMEX through Dr. Carl Oppenheimer. Dr. John Robinson, NOAA, furnished samples of IXTOC I oil which had been separated from relatively unweathered mousse.

Oil was accommodated in seawater for acute toxicity tests by shaking a 1% mixture of Pt. Baker mousse in seawater one hour on an Eberbach shaker. The mixture was allowed to settle for one hour after shaking before the aqueous phase was siphoned off for chemical and biological studies. For fish studies a different method of preparation was used due to the large volumes of seawater needed. A concentrated mixture of oil in seawater was prepared by blending oil and seawater in a Waring blender for about 30 seconds. The concentrated mixture was then diluted with seawater to a con-

centration which was similar to that prepared on the shaker.

A "water soluble" fraction for chemical and biological studies was prepared from the oil-seawater mixture produced on a shaker as previously described. The mixture was filtered twice through glass fiber filters (Whatman GF/C) by gentle suction.

Accommodated or "soluble" oil was extracted from seawater in separatory funnels with three extracts of dichloromethane.

The crude oil was separated from water in mousse samples by addition of an equal volume of n-hexane followed by centrifugation at 10,000 RPM for 20 minutes. This separation indicated that the Pt. Baker mousse contained about 60% water by volume.

Asphaltenes were removed from all samples by precipitation in n-pentane or n-hexane. A known quantity of each sample was fractionated by adsorption on silica gel by usual column chromatograpic techniques. The sample was placed on the column in a small volume of hexane and saturate, aromatic and NSO fractions were eluted with hexane, hexane:benzene (1:1), and methanol respectively. Two column volumes each of hexane and the hexane-benzene mixture were collected and methanol was collected until the eluate was colorless.

Gas chromatography was carried out on a Perkin-Elmer 910 gas chromatograph equipped with a flame ionization detector. Electronic integration of peak area was performed by a Hewlett-Packard 3352B Data System. The glass capillary column utilized for most analyses was OV-101, 11 m x .25 mm ID. Oven temperature was programmed from 70 to 255 C at 3°/minute.

Some analyses were performed on a 27 m OV-101 column programmed from 100 to 260 C at 2°/minute. Combined gas chromatography-mass spectrometry of selected samples was carried out on a Dupont 21-491 mass spectrometer with a Dupont 21-094 B data system.

Fluorescence spectra were determined with a Perkin-Elmer 204-A spectrophotometer. Samples were dissolved in hexane and excited at 265 nm. The emission spectra were recorded from 250 to 600 nm.

RESULTS

The total concentration of oil accommodated in seawater during the various preparations for biological and chemical studies varied from about 25-35 mg/ℓ (PPM). Specific concentrations for each individual preparation were measured and are reported in the appropriate biological section of this report. Approximately 90% of the oil accommodated into seawater was in the form of particles which were sufficiently large to be removed by filtration through glass fiber filters (Whatman GF/C). The "water soluble" portion therefore contained only about 10% or 2-4 PPM.

Results of silica gel chromatographic separations are given in Table 1. The data indicate IXTOC I oil originally contained 52-53% saturates, 34-35% aromatics and 7-8% NSO compounds. The Pt. Baker mousse used for toxicity tests was found to contain a similar percentage of saturates (51%) but a considerably lower percentage of aromatics (26%). The NSO fraction increased to 18% in the Pt. Baker mousse. The Mexican beach samples studied by the Coast Guard and the 1st IXTOC I tarball collected on South Padre Island show similar percentages of silica gel fractions. These percentages are lower in aromatics and higher in NSO compounds than most tarballs collected in a study conducted on St. Joseph and Padre Islands during the past years (Scalan, 1979).

Gas chromatography indicated the saturate fraction of the Pt. Baker mousse (Figure 1A) contained n-alkanes from C_{13} to greater than C_{36} with highest concentrations in the C_{18} to C_{20} range. The mousse sample collected 40 hrs after the blowout (Figure 1B) contained n-alkanes lower than C_{10}

TABLE 1

RESULTS OF SILICA GEL COLUMN CHROMATOGRAPHY
OF IXTOC I SAMPLES

Sample Description	Source	% Saturate	% Aromatic	% NSO
Mousse, collected 40 hrs after blowout	Dr. Botello, Mexico	52.0	34.0	N.A.
Oil, unweathered	Dr. John Robinson NOAA	51.4	31.8	6.9
Mousse, unweathered	Dr. Oppenheimer PEMEX	53.0	34.7	7.5
Mousse, Pt. Baker 22°50'N 96°26'W	U.S.C.G.	51.0	26.4	18.3
Pt. Baker mousse, accommodated particles	UTMSI/PAML	48.0	18.7	19.4
Beached oil, lower beach, Mexico	U.S.C.G.	34.7	28.5	34.1
Beached oil, upper beach, Mexico	U.S.C.G.	38.0	18.0	38.3
Beached oil, mid-beach, Mexico	U.S.C.G.	37.3	14.0	40.5
Tarballs in surf Mexico	U.S.C.G.	29.4	17.3	40.2
Tar on beach, Mexico	U.S.C.G.	36.7	15.8	43.3
"First" IXTOC tarball-South Padre	NOAA	34.4	15.4	39.4
Avg. of non-IXTOC I beach tars, 1978-1979 St. Joseph Island,Tex	Dr. Scalan UTMSI/PAML	46.8 (8-77)	32.3 (14-55)	20.8 (8-41)

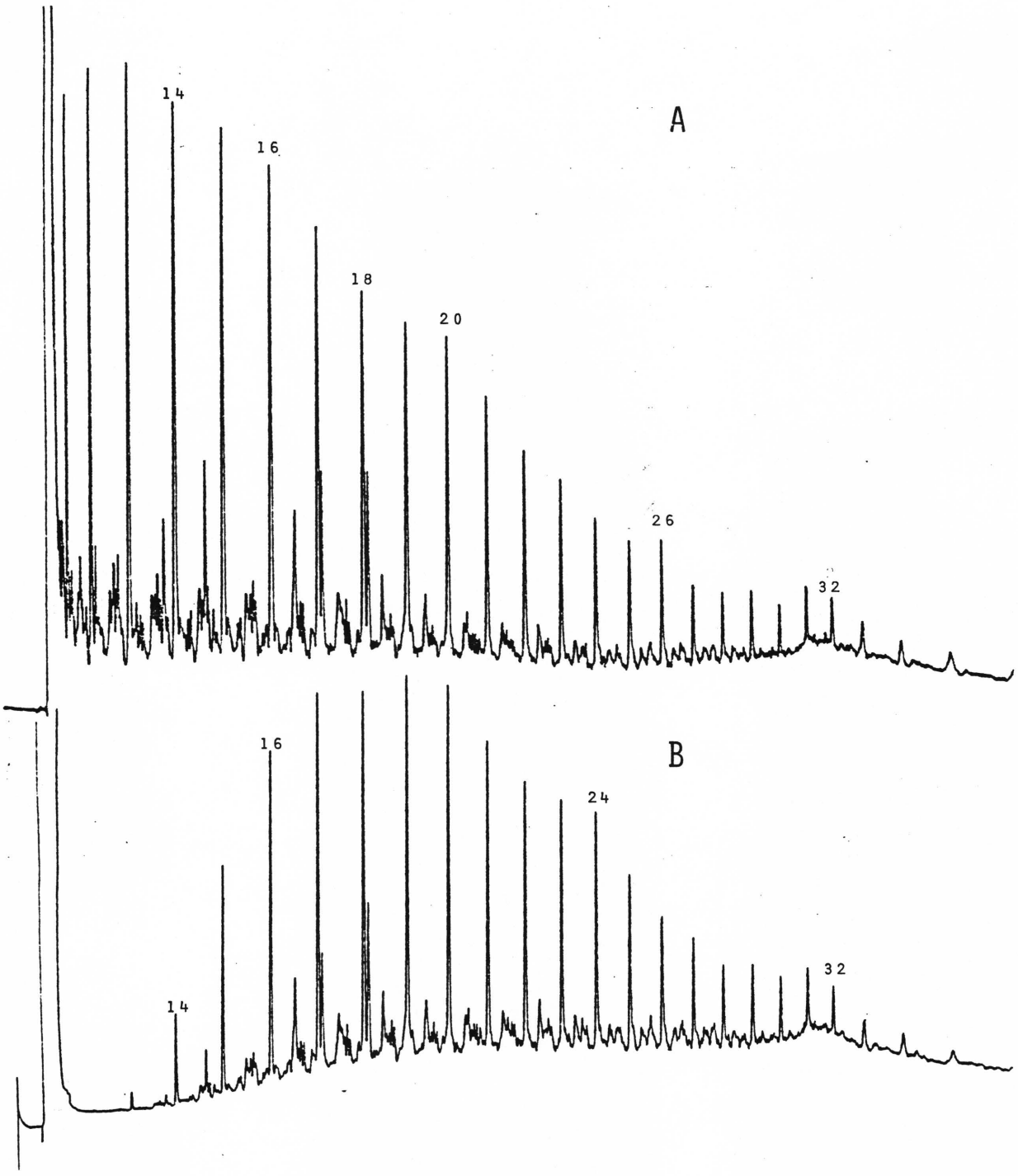

Figure 1. Gas chromatograms of the saturate fraction of a mousse sample collected 40 hrs after the IXTOC I blowout (A), and the Pt. Baker mousse (B).

TABLE 2

GAS CHROMATOGRAPHIC PEAK IDENTIFICATION

N - Naphthalene

MN - Methylnaphthalenes

DMN - Dimethylnaphthalenes (includes ethylnaphthalenes)

TMN - Trimethylnaphthalenes (includes all methylethyl- and propyl-naphathalenes)

DBT - Dibenzothiophene

P - Phenanthrene

MP - Methylphananthrenes

MDBT - Methyldibenzothiophenes

DMP - Dimethylphenanthrenes (see DMN)

DMDBT - Dimethyldibenzothiophenes (see DMN)

with the maximum at C_{13}.

Analysis of the aromatic fraction from the Pt. Baker mousse (Figure 2A) revealed the presence of alkyl naphthalenes, primarily C_2-(dimethyl+ethyl) and C_3 homologs. C_3-naphthalenes were present at a concentration which was about one-third of that present in the 40 hr sample (Figure 2B). Three ring aromatic compounds such as phenanthrenes and dibenzothiophenes are present in both the Pt. Baker mousse and the 40 hr sample in similar relative concentrations. The IXTOC I oil contains a relatively high concentration of alkyl dibenzothiophenes with concentrations similar to those of alkyl phenanthrenes.

Analyses of the oil accommodated into seawater for biological testing indicated that the particulate oil which was removed by filtration had a saturate and aromatic composition which was quite similar to the Pt. Baker mousse.

The "water soluble" material from the accommodated oil-seawater mixture was not fractionated on silica gel. A typical chromatogram of the "water solubles" is shown in Figure 3. The increased concentration of more soluble aromatic compounds relative to the Pt. Baker mousse was evident. The presence of n-alkanes in the "water soluble" fraction probably indicates that some oil particles were sufficiently small to pass through the glass fiber filters. The n-alkane distribution in the soluble fraction was from about C_{19} to greater than C_{37} with a maximum at about C_{31}. This distribution was significantly different from the Pt. Baker mousse which had a maximum at about C_{19}.

The samples of oil from a Mexican beach gave similar results with some samples showing slightly more weathering. Typical chromatograms of saturate and aromatic fractions are given in Figure 4. Normal alkanes began at C_{15} with a maximum at C_{21}. The aromatic fraction indicated that alkyl naphtha-

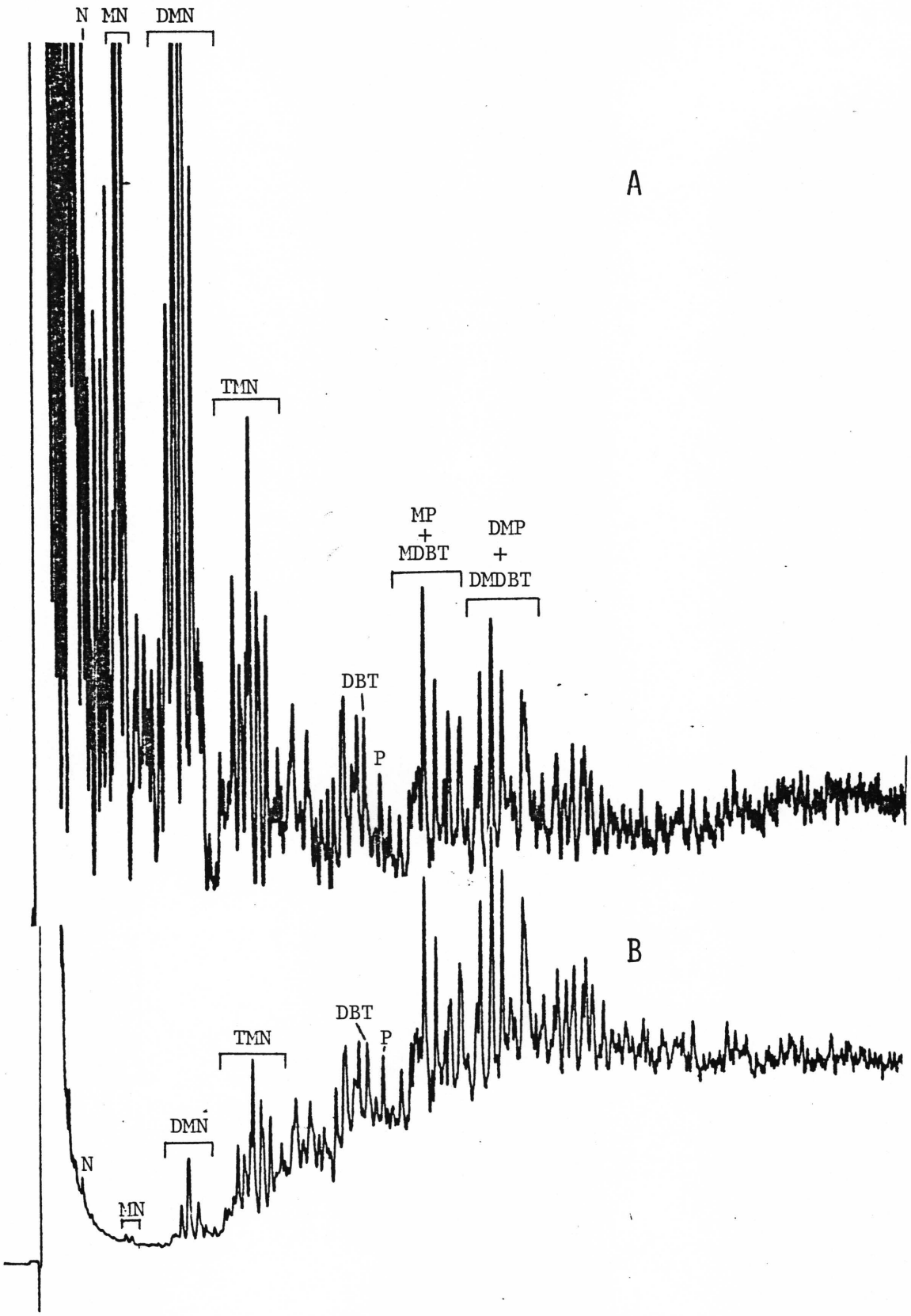

Figure 2. Gas chromatograms of the aromatic fraction of a mousse sample collected 40 hrs after the IXTOC I blowout (A), and the Pt. Baker mousse (B). (See Table 2 for peak identifications.)

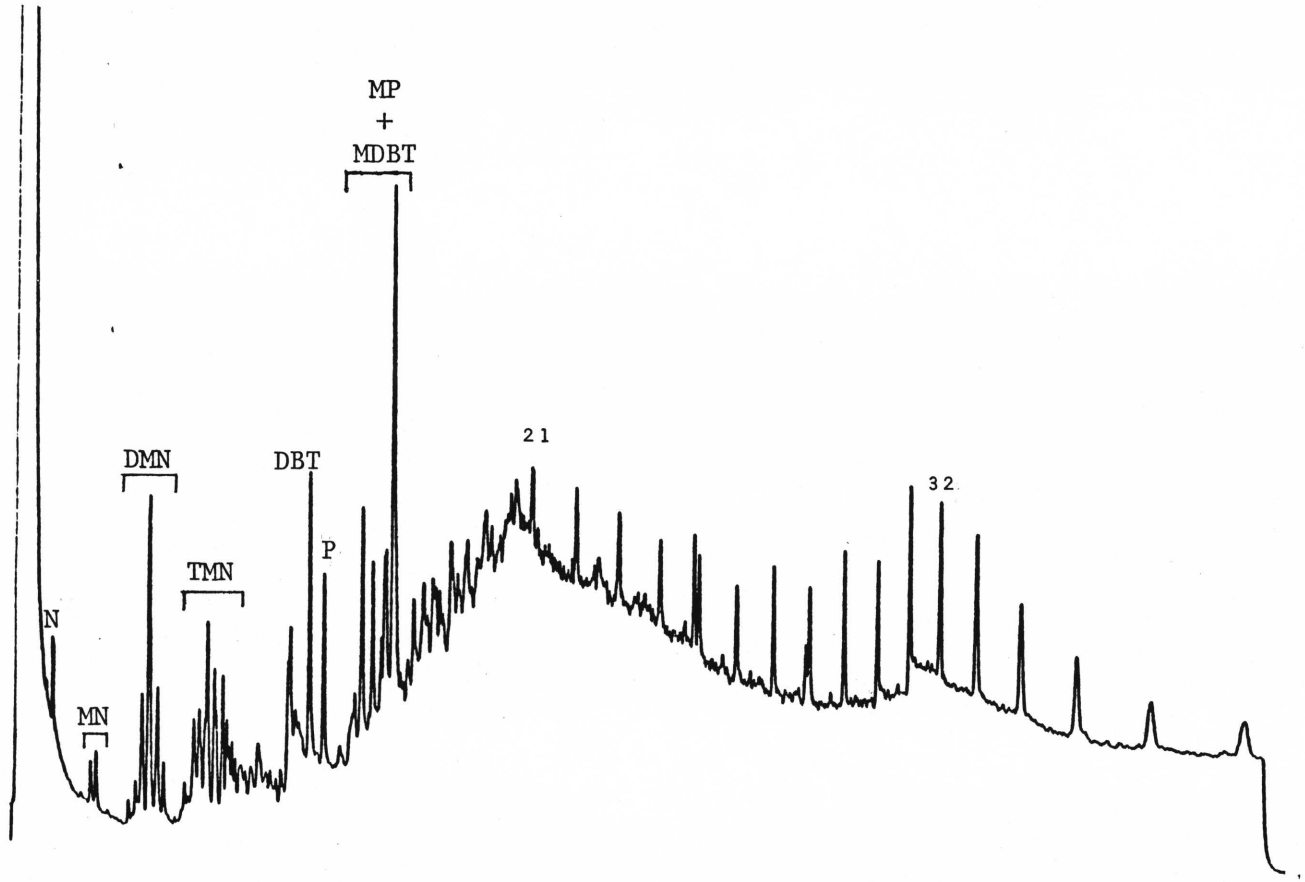

Figure 3. Gas chromatogram of the "water soluble" fraction prepared from the Pt. Baker mousse. (See Table 2 for peak identification.)

Figure 4. Typical chromatograms of the saturate (A), and aromatic (B), fractions of IXTOC I oil from a Mexican beach. (See Table 2 for peak identification.)

lenes had been almost completely lost. Phenanthrene, dibenzothiophene and methyl homologs were also significantly lower in concentration relative to the C_2 and C_3 homologs.

A chromatogram of the saturate fraction of the tarball from South Padre Island is shown in Figure 5A. Maximum n-alkane concentration occurred at C_{20}. The aromatic fraction (Figure 5B) was found to contain a large concentration of alkyl benzenes and naphthalenes relative to all other aromatic fractions analyzed other than samples of very fresh mousse. A chromatogram of the aromatic fraction of Pt. Baker mousse has been included in Figure 5C for comparison.

Combined gas chromatography-mass spectrometry was used to verify identification of major components in saturate and aromatic fractions of representative samples. Mass chromatograms as shown in Figure 6 and 7 for alkyl naphthalanes and phenanthrenes, respectively, were used routinely.

Fluorescence emission spectra of several samples are presented in Figure 8.

DISCUSSION

Chemical analyses indicated the Pt. Baker mousse used for biological studies had been sufficiently weathered at sea to significantly alter the composition of the saturate and aromatic fractions. Virtually all alkyl benzenes and a large percentage of the two ring aromatics, primarily naphthalenes, had been removed. Three ring compounds such as phenanthrenes and dibenzothiophenes, however, were quite similar in concentration to that present in samples of unweathered IXTOC I oil.

Unlike the Pt. Baker mousse the tarball collected on South Padre Island contained a high percentage of low molecular weight aromatics including alkyl benzenes and naphthalenes. Analyses of a suite of tarballs collected

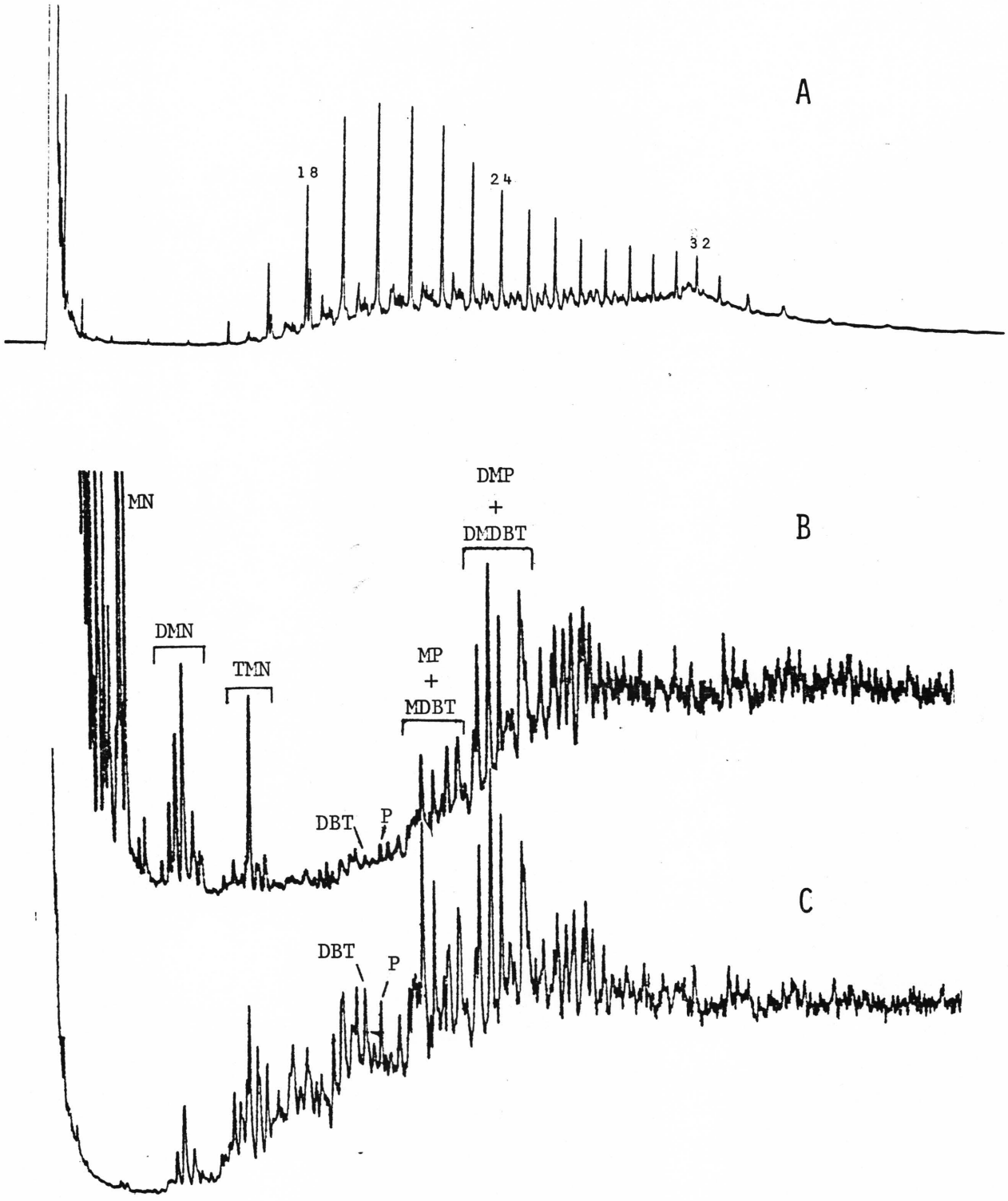

Figure 5. Gas chromatogram of the saturate (A), and aromatic (B), fractions of an IXTOC tarball from South Padre Island, Texas. Pt. Baker mousse aromatic fraction (C), shows much lower volatile aromatics. (See Table 2 for peak identification.)

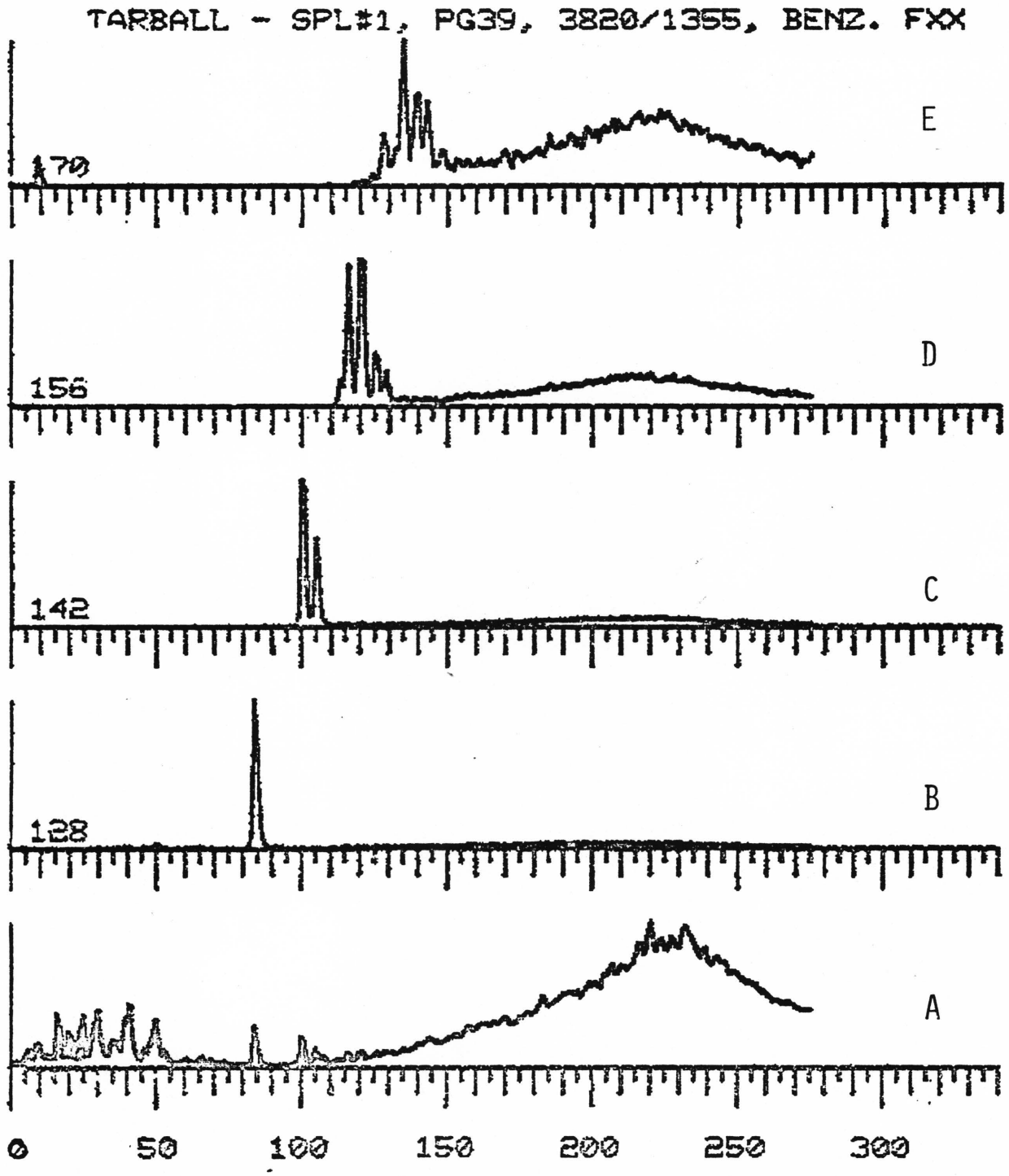

Figure 6. Mass chromatograms of the aromatic fraction from the South Padre Island tarball sample. Total ion chromatogram (A); m/e=128 naphthalene (B); m/e=142, methylnaphthalenes (C); m/e=156, C_2-naphthalenes (D); m/e=170, C_3-naphthalenes (E).

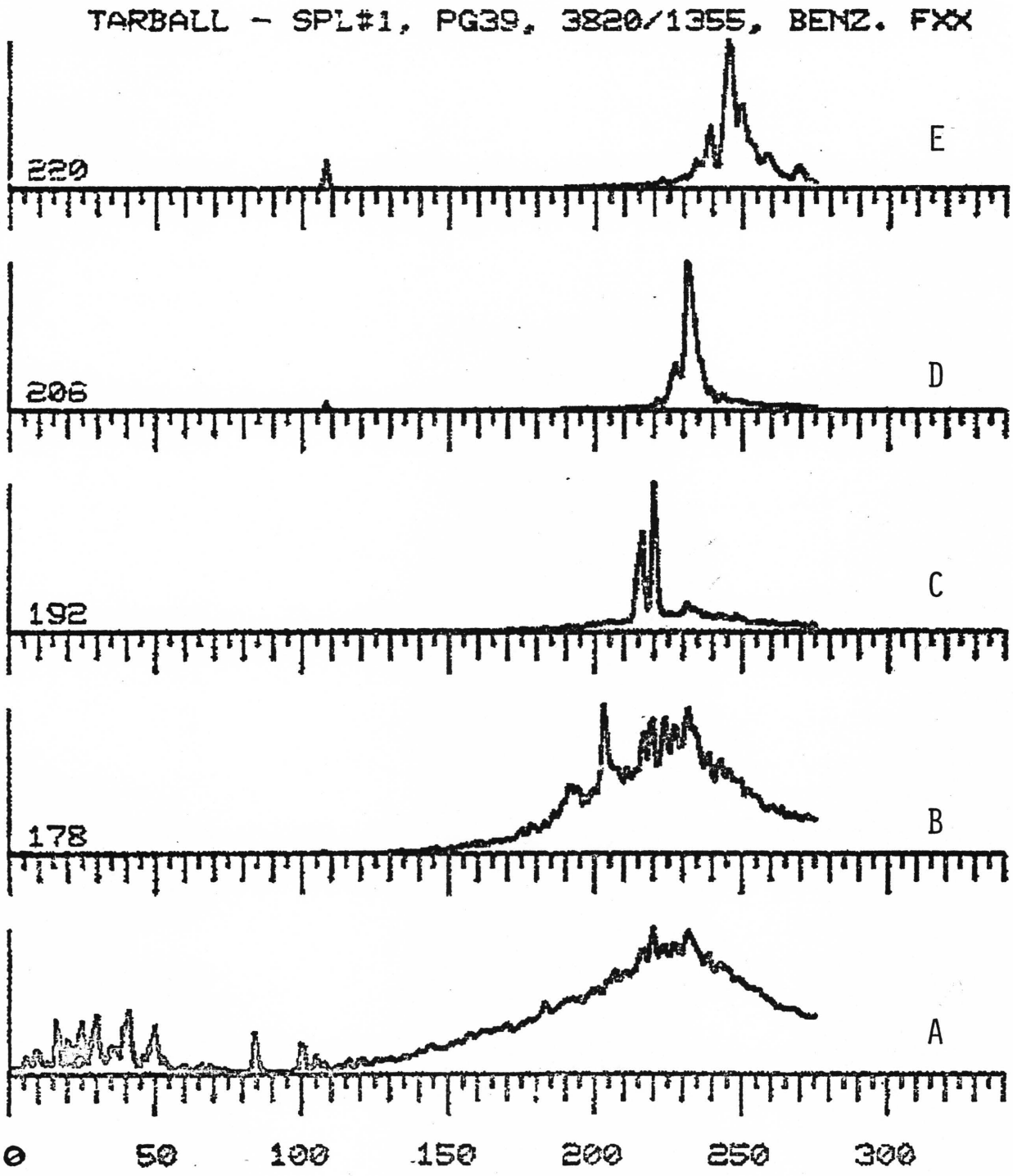

Figure 7. Mass chromatograms of the aromatic fraction from South Padre Island tarball sample. Total ion chromatogram (A); m/e=178, phenanthrene (B); m/e=192, methylphenanthrenes (C); m/e=206, C_2-phenanthrenes (D); m/e=220, C_3-phenanthrenes (E).

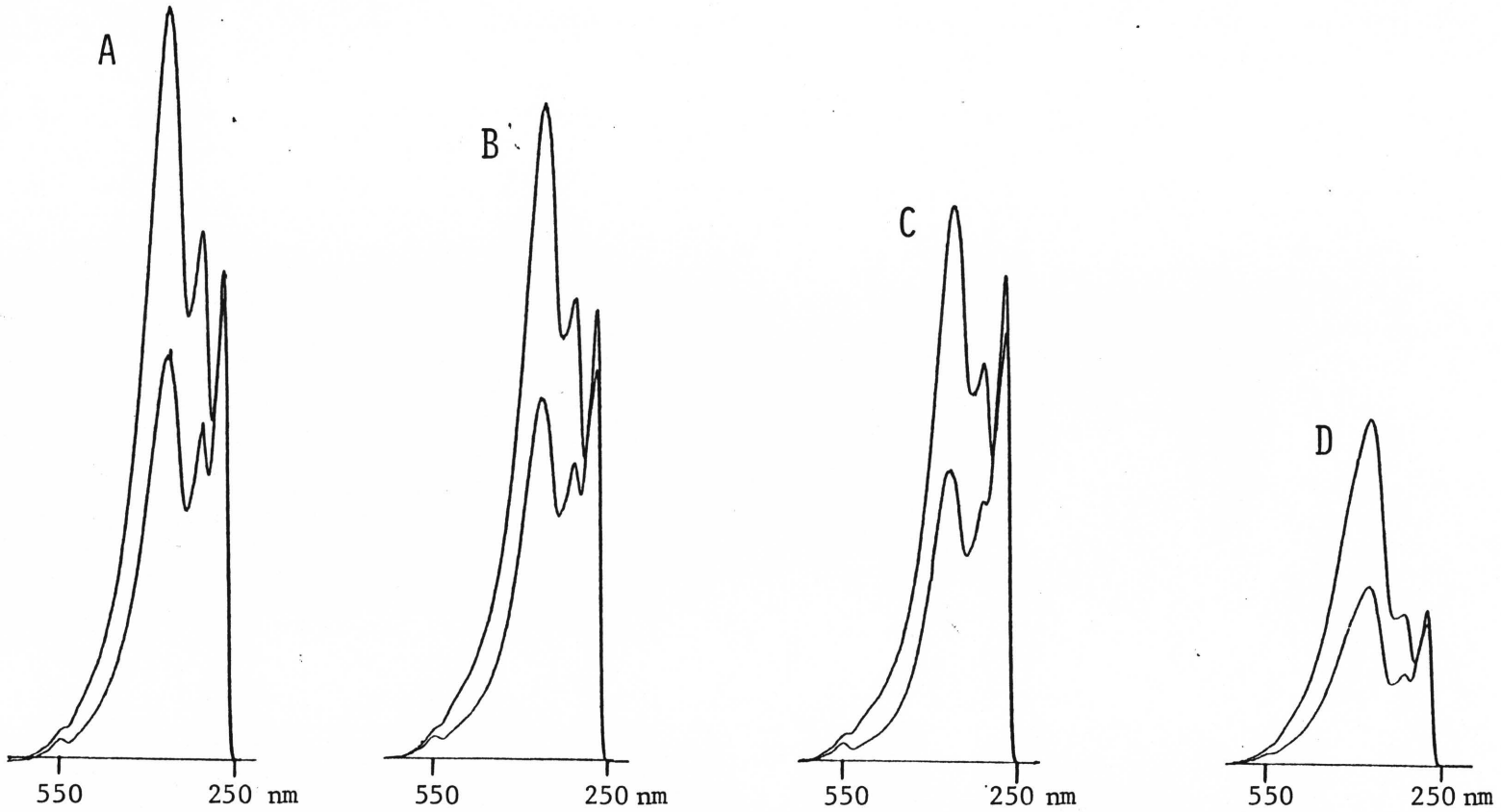

Figure 8. Fluorescence emmission spectra of IXTOC I oil samples A, B, C and a non-IXTOC tarball (D). Exitation was at 265 nm. Lower trace represents spectrum obtained at one-half concentration.

on the recent MOUSSE I cruise of the R/V LONGHORN indicated that indeed most floating tarballs contained alkyl benzenes and naphthalenes in proportions similar to the South Padre Island sample.

The differences in chemical composition between the Pt. Baker mousse and the tarballs raise two important questions. The first question is whether or not the Pt. Baker mousse is representative of the large amount of oil which is impacting the various biological communities and therefore the validity of biological results based on this sample. A second question relates to the processes by which tarballs are formed from the IXTOC I oil. If the mousse formed at the well site has drifted north and slowly disintegrated into smaller patches (pancakes) and ultimately into tarballs, as many people have speculated, how can such a high proportion of alkyl benzenes and naphthalenes be present in tarballs and not in an intermediate mousse (Pt. Baker)?

Two additional subsamples of the Pt. Baker mousse were analyzed with special care to avoid loss of volatile components. The results of both subsamples indicated an absence of alkyl benzenes and low concentrations of naphthalenes similar to the previous analysis.

Chromatograms of aromatic fractions of the Pt. Baker mousse and South Padre Island tarball analyzed on a 27 m OV-101 column are given in Figures 9 and 10, respectively.

Greater resolution on the longer columns should be useful for further more detailed studies of changes in composition during weathering. The large amount of alkyl benzenes and naphthalenes present in the tarball (Figure 10) was even more evident on the longer column.

The fluorescence analyses were undertaken to develop a quick screening technique which would indicate with some certainty that a given oil, mousse,

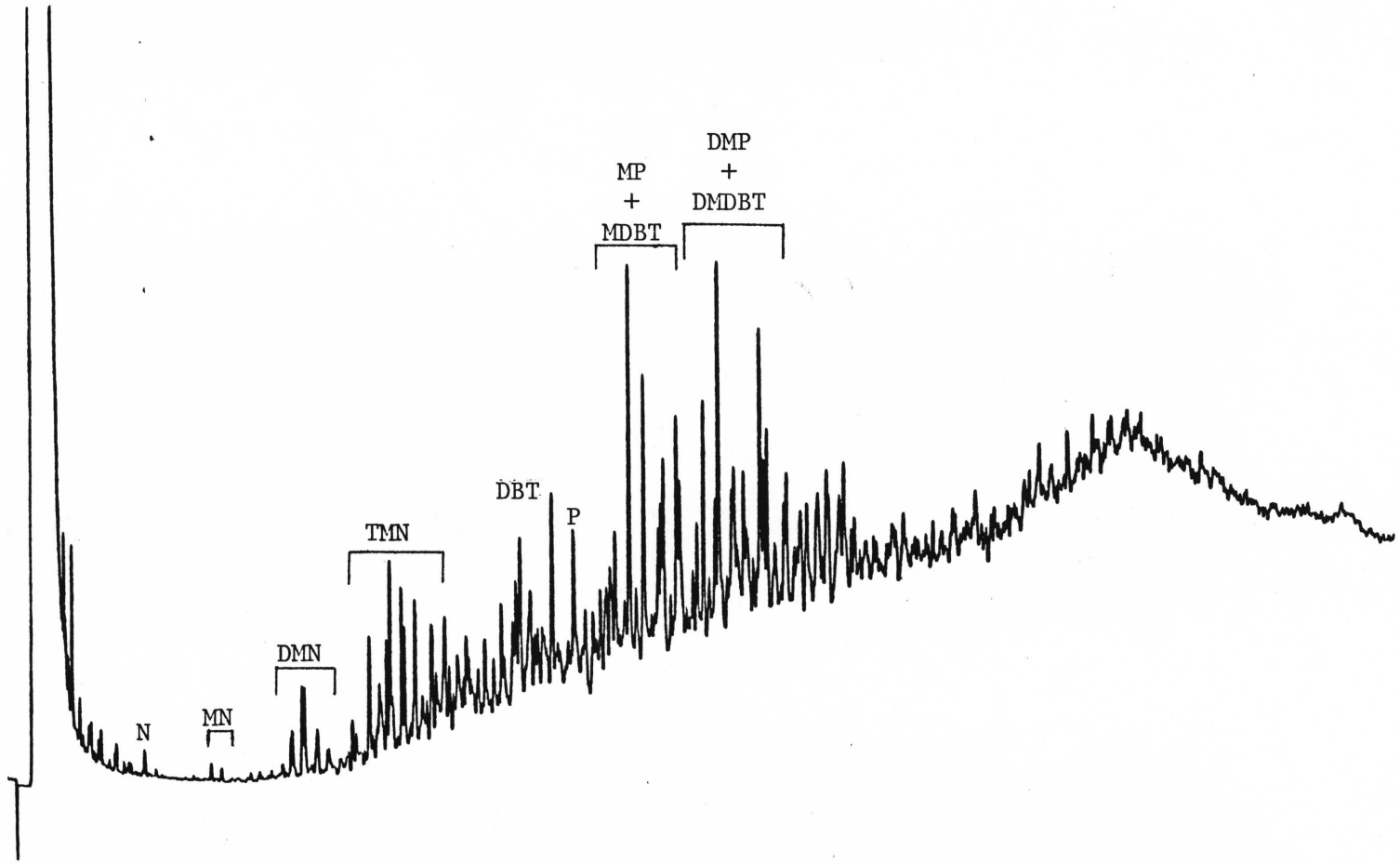

Figure 9. Gas chromatogram of the aromatic fraction from Pt. Baker mousse on a 27 m OV-101 column. (See Table 2 for peak identification.)

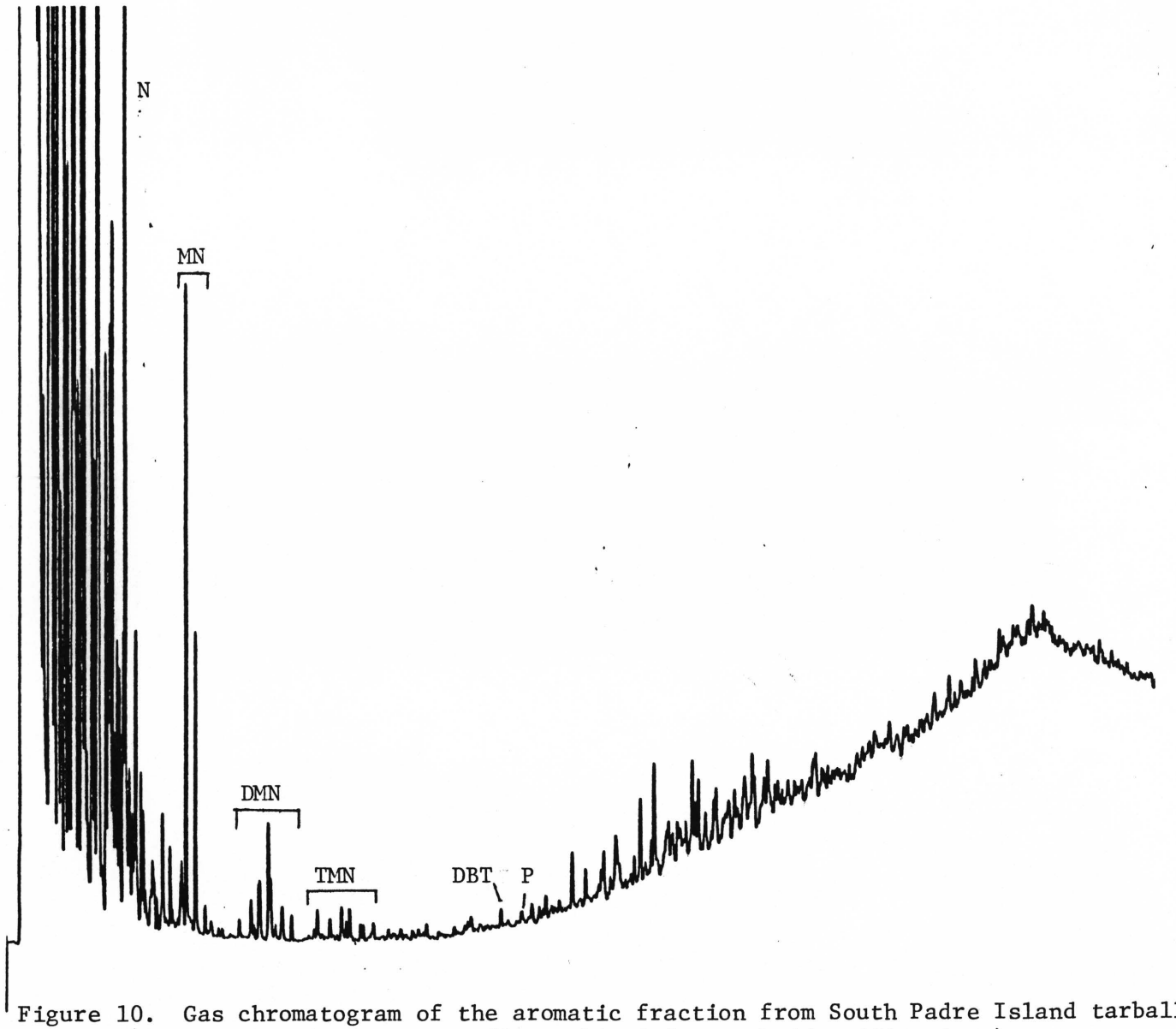

Figure 10. Gas chromatogram of the aromatic fraction from South Padre Island tarball on 27 m. OV-101 column. (See Table 2 for peak identification.)

or tarball was in fact from IXTOC I.

The method can also be used to give a semi-quantitative estimate of the amount of oil present in a sample such as a sediment. The rate at which various weathering processes will alter the emission spectra and reduce the effectiveness of this procedure is currently being investigated.

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SECTION B

EFFECT OF MEXICAN OIL ON PHYTOPLANKTON AND SEAGRASS PHOTOSYNTHETIC ACTIVITY AFTER SHORT-TIME EXPOSURE

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INTRODUCTION

An attempt was made to assess acute toxicity of Mexican Oil to mixed phytoplankton populations and seagrasses by comparing photosynthetic rates in the presence and absence of a seawater-soluble fraction or oil-accomodated seawater preparation. This type of measurement is by nature a short-term bioassay, which can only indicate whether the material being tested has an immediate effect on photosynthesis of the cells involved. Several studies have shown that seawater equilibrated with No. 2 fuel oil (Pulich *et al.*, 1974; Gordon and Prouse, 1973) and some crude oils (Lacaze and deNaide, 1976) is inhibitory in varying degrees to photosynthesis of microalgae (*e.g.* blue-greens, greens and diatoms) and mixed phytoplankton samples. While the effect from No. 2 fuel oil was immediate, the crude oil response required an exposure period of several days to a week during which time chemical and photochemical modification of the oil apparently takes place. Thus, No. 2 fuel oil can be described as significantly more toxic than the crude oils tested in this manner.

MATERIALS AND METHODS

Seawater samples were collected at the South Jetty in Port Aransas (prior to oil spill). The natural phytoplankton population was concentrated within two hours after collecting by centrifugation at low speed (1000 xg) for 20 minutes. The mixed phytoplankton were resuspended in 10-15 ml fresh seawater for photosynthesis measurements. Oxygen evolution and consumption were followed using a sensitive Clark-type oxygen microelectrode system as described by Pulich *et al* (1974). Soluble extracts of the oil were prepared by shaking approximately 150 g oil with one liter offshore seawater for two hours and then filtering through a 0.4 μm glass-

fiber filter after two hours. Toxicity of the oil was determined by comparing the effect of seawater-soluble extracts on the concentrated mixed phytoplankton to unexposed controls. Samples were exposed to 100% seawater soluble material (WSF).

Seagrass photosynthesis was measured by $H^{14}CO_3$ uptake as described by Pulich *et al* (1976) with one modification: HCO_3 was present at a saturating level (ca 200 mg/ℓ). Oil-accomodated seawater (*i.e.* the unfiltered seawater phase described above) was tested.

RESULTS

Phytoplankton Bioassay

Two runs were made with mixed microplankton samples to test the immediate effect of water soluble fraction on photosynthetic activity. In this case, the concentrated phytoplankton were not exposed to the oil until oxygen measurements were started and then for only about 30 minutes total. The results presented in Table 1 indicate no significant change in photosynthetic rate compared to controls for either set of samples. Oxygen evolution proceeded at a rate of ca 5.0 µℓ O_2/µg chl/hr for Run I and ca 2.7 µℓ O_2/µg chl/ hr for Run II. These rates were measured at saturating and also two-fold higher than saturating light intensity. Respiration for two minutes after photosynthesis was variable (in Run I even higher in the WSF-treated sample than control), but did not appear decreased. In these two runs, the algae consisted of diatoms, coccoid greens or blue greens, and green flagellates by cursory microscopic examination.

A further test of acute toxicity was carried out by exposing mixed microplankton samples to WSF for up to eight hours in dim light. However, oxygen measurements at eight hours showed no significant differences in oxygen evolution between controls and treated samples, as shown by a typi-

Table 1. Effect of seawater-soluble fraction from Mexican oil on oxygen production and consumption by mixed phytoplankton samples. Values are averages of 2-3 measurements ± range, expressed in $\mu\ell\ O_2 \cdot \mu g\ chl\ a^{-1} \cdot hr^{-1}$

Sample	EXPERIMENT I		EXPERIMENT II	
	Photosynthesis	Respiration	Photosynthesis	Respiration
Control	5.16 ± 0.58	2.58 ± 0.30	2.70 ± 0.23	2.33 ± 0.22
Treated with 100% WSF	4.92 ± 0.40	7.50 ± 0.64	2.58 ± 0.30	2.25 ± 0.16

cal oxygen electrode trace after eight hours exposure (Fig. 1). Photosynthesis in this experiment was measured at 2.2 ± 0.3 μℓ O_2/μg chl/hr. Respiration, however, showed a significant decrease of ca 20% in the WSF-treated samples (4.8 in WSF sample compared to 6.0 μℓ O_2/μg chl/hr in control). This was particularly noticeable for 2-3 min. after photosynthesis, although endogenous respiration also appeared depressed. This experiment was performed with water samples containing very little particulate matter (chl = 0.18 μg/ℓ), typical of offshore water. Microscopic examination showed mostly diatoms and green flagellates present.

Seagrass Bioassay

Intact leaves of *Halodule* and *Halophila* were in direct contact with oil microdroplets over the course of photosynthetic $H^{14}CO_3$ fixation, including one hour in the dark prior to incubation in the light for 6-7 hrs. The total quantity of oil present in the incubation bottle was 2.9 mg organic C per 68 ml seawater present. The data in Tables 2 and 3 suggests strongly that the oil emulsion at this concentration has little immediate effect on carbon fixation of both *Halodule* and *Halophila*. The somewhat lower rates for oil-treated *Halodule* at low and medium light intensity are attributed to shading of leaves by the "cloudiness" imparted to the incubation seawater by abundant oil droplets. At the highest light intensity, oil-treated *Halodule* had achieved the equivalent rate of photosynthesis as the control. For *Halophila* (Table 3) there was no difference between control and oil sample at medium light intensity.

DISCUSSION

These photosynthesis bioassays can be considered as evidence that the weathered Mexican oil (in "mousse" form) did not immediately inhibit photosynthesis of representative nearshore phytoplankton samples and seagrasses.

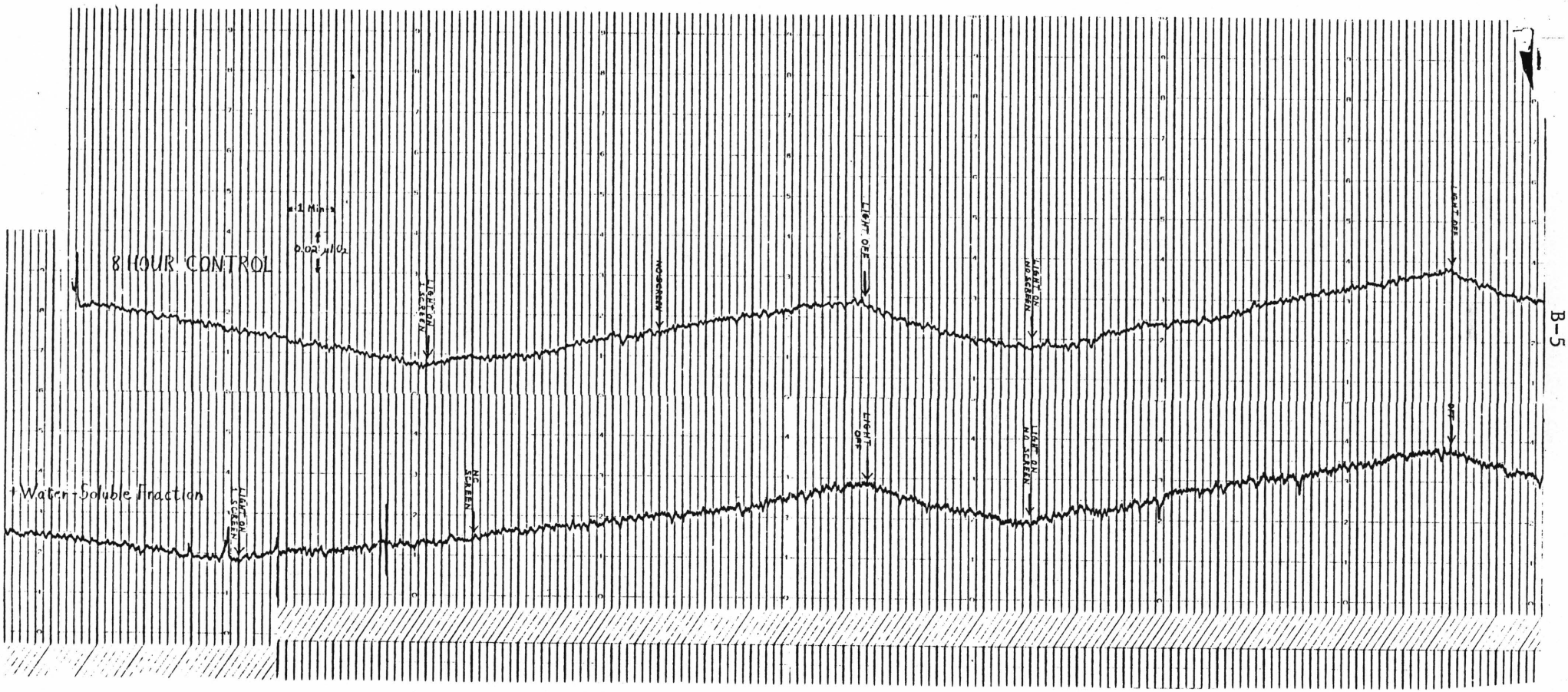

Figure 1. Typical oxygen electrode trace showing oxygen production and consumption for mixed natural phytoplankton samples after 8-hr exposure to seawater-soluble fraction of Mexican oil.

Table 2. Effect of oil-accommodated seawater on photosynthetic activity* of the seagrass, *Halodule wrightii*, measured at 30°C and three light intensities. Rates determined by $H^{14}CO_3$ uptake over 6-7 hrs. Corrected for dark uptake.

Light Intensity	Control	Oil-Treated
LOW	9.6 ± 0.1	7.9 ± 0.2
MEDIUM	13.0 ± 0.6	11.0 ± 0.2
HIGH	12.0 ± 1.0	13.0 ± 0.3

*Values in µg C/mg dry wt/hr; approximately 3.5 µg chl a per mg dry weight

Table 3. Effect of oil-accommodated seawater on photosynthetic rate of seagrass, *Halophila engelmannii*, measured at 30°C and medium light intensity. Rates determined by $H^{14}CO_3$ uptake over 6 hr.

Sample	µg C/mg dry weight/hr
Control	18.0 ± 1.0
Oil-treated	19.0 ± 2.0

This information, however, should not be interpreted as evidence that the Mexican oil will have no effect (either inhibitory or enhanced) on growth and cell division of the plant species involved. Winters *et al* (1977) have pointed out that seawater extracts of fuel oils are quite varied in their immediate effects on photosynthesis. For example, though growth of both blue-green algae and some green algae was inhibited in the presence of New Jersey fuel oil water solubles, only the green algae, but not the blue-green, showed inhibition of photosynthesis. Moreover, these workers also found that pure compounds such as p-toluidine (selectively toxic to blue-greens) and phenalen-1-one (toxic selectively to green algae) which are lethal to the algae mentioned, had no immediate effect on oxygen evolution. Thus, it would not be safe to extrapolate these results to growth of entire Gulf of Mexico phytoplankton populations! At best, we may say that the Mexican oil does not appear to be as toxic as a No. 2 fuel oil. Experiments involving growth rate measurements should be carried out. This same recommendation would apply to seagrass growth as well, particularly since no information at all exists on effects of petroleum on seagrasses.

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SECTION C

EFFECTS OF MEXICAN CRUDE OIL ON REDFISH (*Sciaenops ocellata*) EGGS AND LARVAE

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INTRODUCTION

The loss of important marine resources through pollutants such as crude oil has become an area of increasing concern. The recent blowout of IXTOC I in the Gulf of Campeche, Mexico, has made it necessary to evaluate the extent to which this disaster could affect Texas fisheries. The redfish (*Sciaenops ocellata*) is one of the most important commercial and sport fishes in Texas waters. Redfish generally spawn around the mouth of tidal inlets on the Texas coast in the late summer and early fall. Since oil from the IXTOC I may be in Texas waters while redfish are spawning and since the early stages in the life cycles of most organisms are usually considered to be those most susceptible to deleterious perturbations, it was decided to ascertain the effect of Mexican crude on redfish eggs and larvae.

METHODS

In order to determine the toxicity of Mexican crude oil on the eggs and newly hatched larvae of the redfish, three tests were conducted using three forms of Mexican crude. In all cases the eggs and larvae were obtained from captive redfish which were induced to spawn using light and temperature manipulation to simulate natural conditions. All tests were performed in 32 ppt seawater, maintained at 26°C.

In the first of these tests, a 1% solution (25-30 ppm) of oil accommodated water (OAW) was used. This solution was prepared by agitating Mexican crude oil and filtered seawater for two hours and then allowing it to settle for two hours. The water at the bottom of the container which was relatively free of large oil globules was decanted off and used as OAW. Concentrations of 0-100% seawater/OAW were prepared. Fifty (50), 1-day

old redfish eggs were placed in each of the 11 different concentrations and allowed to stand without aeration. After 24 hrs the number of live larvae were counted.

The second experiment was conducted in the same manner as the first with the following exceptions. The OAW was filtered using a .45 μ millipore filter and the water soluble fraction (WSF) was retained. Twenty-five (25) 1-day old redfish eggs, from a different spawn than were used in Experiments 1 and 3 were placed in mixtures of 0% and 50-100% WSF and filtered seawater. The number of live and dead larvae and unhatched eggs were recorded.

In the third experiment, 500 16-hr old redfish eggs were placed in 1 ℓ of unfiltered seawater to which 4 g of Mexican crude oil had been added. The mixture was maintained with gentle aeration. Another mixture of 9 g of mousse to 1 ℓ of water was prepared, aerated vigorously, and maintained at room temperature. Five hundred (500) 1-hr old redfish eggs were added and the number of live larvae were counted after 24-hrs. Live and dead larvae were counted in the 4 g preparation, and live larvae were counted in the 9 g preparation.

Experiments using mousse were repeated one week later. Five hundred (500) 14-hr old eggs were placed in each of three 1 ℓ beakers and gently aerated. One beaker was not treated with oil and acted as a control. Nine grams of mousse were placed in one of the remaining beakers, and 4 g were added to the other. After 24 hrs the total number of live larvae, live deformed larvae, dead larvae and unhatched eggs were counted. Deformity was defined as the body bent dorsally and/or a large unabsorbed yolk sac.

RESULTS

Only live larvae were counted in Experiment 1 (Table 1). It was therefore impossible to ascertain the affect of the OAW on redfish eggs.

TABLE 1

SURVIVAL OF REDFISH LARVAE AFTER 24 HRS IN OAW*

% OAW		# LIVE LARVAE	% MORTALITY	
0	(Control)	31	38	
10		26	48	
20		17	66	
30		13	74	
40		13	74	
50		7	86	
60		9	82	
70		8	84	
80		7	86	← LD50
90		5	90	
100		5	90	

* oil accommodated water

Hatch percentage of nearly 100% were observed in other eggs from this same spawn. This along with the short time (< 1 hr) which the eggs were subjected to the OAW before hatch indicates the 38% mortality in the control (0%) is an expression of normal mortality in 24-hr old redfish. The control mortality (38%) was subtracted from the observed mortality in the remaining concentrations to obtain LD_{50}. LD_{50} was experienced between 80 and 90% OAW/seawater mixture. LD_{50} was also approached at 50% OAW/seawater.

Only live and dead larvae were used to determine LD_{50} in Experiment 2 (Table 2). No dead larvae were found in the control or 50% WSF. LD_{50} was experienced between 60 and 70% WSF.

A large percentage (80%) of the 127 live larvae treated with 4 g of Mexican mousse in Experiment 3 were either deformed, unresponsive to tactile stimuli or moved very slowly. There was a large number (250) of dead larvae which were highly deformed. Many of the larvae were brown in color and retained a large yolk sac indicating that death occurred very shortly after hatching. After 24 hrs only five live larvae were found in the 9 g mousse/water mixture. Many of the unhatched eggs were observed floating in mousse which had accumulated at the water surface.

In the repeat of the mousse experiment using 14-hr eggs, the total number of eggs and larvae which were accounted for after 24 hrs. was lowest in the highest oil concentration (Table 3). This was probably due in part to the fact that unhatched eggs were entrapped in the mousse and were not counted. Mortality was highest in the 9 g treatment where 95% of the larvae accounted for were dead or live deformed. Seventy-seven (77) percent of the larvae accounted for in the 4 g treatment were dead or live deformed. After 48 hrs all larvae in the oiled beakers were dead. Live fish were still observed in the control after 48 hrs.

TABLE 2

SURVIVAL OF REDFISH EGGS AND LARVAE AFTER 24 HRS IN WSF*

% WSF*	LIVE LARVAE	DEAD LARVAE	UNHATCHED EGGS	% MORTALITY
0(Control)	16	0	7	0
50	22	0	3	0
60	17	2	4	11
70	6	16	2	73 ← LD50
80	8	14	2	64
90	6	19	0	76
100	2	19	0	94

*Water soluble fraction

TABLE 3

SURVIVAL OF REDFISH EGGS AND LARVAE AFTER 24 HRS

IN MEXICAN CRUDE OIL MOUSSE /SEA WATER

GRAMS OF MOUSSE /1 l SEAWATER	LIVE	LIVE DEFORMED	DEAD	UNHATCHED EGGS
0 g (Control)	298	17	115	15
4 g	32	394	18	20
9 g	3	89	214	8

CONCLUSIONS

The water soluble fraction (WSF) of Mexican crude oil was more toxic than Mexican crude oil accommodated water (OAW) because LD_{50} occurred at a lower concentration in the WSF than in the OAW. The mousse at 9 g/ℓ water was more toxic than a mousse mixture of 4 g/ℓ water. The large number of deformed larvae in the lower concentration of mousse indicated that possibly even this mixture would cause 100% mortality after an extended period.

SECTION D

A TOXICITY STUDY OF IXTOC I OIL FOR ZOOPLANKTON

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ABSTRACT

The Mexican IXTOC oil well blew out on June 3, 1979. Since then more than 1.7 million barrels of crude oil have been spewed into the Gulf of Mexico. This is the largest oil spill in the world's history. Two months later (August 7, 1979), the spilled oil began moving to the Texas coastal waters and the well was not yet capped. Although laboratory tests showed that the aged oil was not acutely toxic to some marine invertebrates, care still should be taken to test its chronic sublethal effects and the other potential damages to the environmental sensitive ecosystem such as the Laguna Madre, a nursery ground for many shrimp and fish.

INTRODUCTION

The oil well, IXTOC I, being drilled in the Bay of Campeche, Mexico, blew out 3 June 1979. Since then more than 1.7 million barrels of crude oil have entered the Gulf of Mexico. Portions have been moving northward and recently reached the Texas coast. This study, a part of an acute toxicity program, was devoted to a laboratory toxicity assessment regarding selected small invertebrates.

Two series of experiments were carried out on the acute toxicity of oil accommodated in seawater (OAS), made from the spilled Mexican oil. In one experiment, mixed natural zooplankton were immersed in the OAS for 96 hours. A vital staining method was employed to distinguish the dead from live individuals. In another experiment, a subtidal amphipod, *Parhyale hawaiensis* (Dana) was exposed to the OAS for one week. Mortality was determined daily during the experiment. Values of 96-h-LC_{50} were estimated by the method described by Litchfield and Wilcoxon (1949) whenever data were sufficient.

METHODS AND MATERIALS

Juvenile amphipods (1 month old) used in the toxicity study were from our stock culture which has been kept in the laboratory since 1976. They were kept in glass fiber filtered seawater (salinity 30 ‰) and fed with ground dry algae, *Ulva*, and tropical fish food flake.

Zooplankton were collected at the Marine Laboratory pier. They were acclimated in seawater of 30 ‰ and room temperature of 24°C for two days before the experiments were started. Zooplankton were fed with a mixture of algae comprised of *Dunaliella tertiolecta* and *Isochrysis galbana*.

Oil was layed on the top of filtered seawater in a 4 ℓ aspirator bottle with a tube outlet near the bottom and shaken on an Eberbach Shaker at low speed (260 excursions/min) for two hours. The lower portion, oil accommodated in seawater (OAS) was drained after a two hour settling period and used as a stock OAS.

The chemical composition and the total amount of oil suspended and dissolved in seawater were analyzed at the time the experiment began. The stock OAS thus prepared contained 27 ppm of oil. Therefore, a 50% dilution would have about 14 ppm of oil. Further details on oil composition were described in the paper by Winters *et al* in this report.

In addition to the control, juvenile amphipods were exposed to the following dilutions of OAS; 50, 40, 30, 20, 10 and 1%. At each concentration, 40 individuals were divided into two groups and added to 200 ml dilutions of OAS. Mortality was observed daily for seven days. Test medium was gently bubbled with air and not renewed during the experimental period.

Natural zooplankton were treated the same way as *Parhyale hawaiensis*; duplicate samples were run for each of the following concentrations of 40, 30, 20, 10 and 1% and control (0%). At the end of 96 hours exposure, 10 ml of neutral red was added to each jar which contained 1 ℓ of test medium and the zooplankton were then fixed following the procedures suggested by Crippen and Perrier (1974). To obtain percentage of survival for each sample, at least 300 individuals were counted, identified and determined for their status of live or dead animals.

All experiments were maintained under the conditions of room temperature 24 ± 2°C and at salinity of 30 ‰. Animals were also fed daily during the experiments.

RESULTS

Parhyale hawaiensis

At all test concentrations, ranging from 0 to 50% OAS, all individuals survived at the end of seven days exposure, except that two amphipods were found to be dead at day seven in 40% OAS (Table 1). Surviving amphipods were actively moving about and no signs of abnormal behavior were observed.

Mixed Marine Zooplankton

Zooplankton used in the toxicity study were typically an assemblage of Texas coastal water species recorded during summer. The calanoid copepod, *Acartia tonsa*, comprised 65% of the test population and the remaining four abundant species in order were *Paracalanus crassirostris*, *Oithona colcarva*, *Corycaeus amazonicus* and *Eucalanus monachus*. These four species together with *A. tonsa* make up more than 95% of test zooplankton populations (Table 2).

Survival of copepods at all test concentrations were high, being $\geq$ 75% (Table 3). No significant trend in mortality can be related to the concentration of OAS tested. High mortality of copepods were recorded in sample 1 of both the control and 1% OAS. The reasons for this were not apparent, but judging from the mortality at the higher concentrations of OAS, we believe that it is more likely caused by some unknown artificial factors rather than by the toxicity of oil.

DISCUSSION

These two studies indicated that the OAS of the spilled Mexican oil was not acutely toxic to the two kinds of crustaceans tested, possibly because this oil has been weathered for a long time. Thus the most toxic

TABLE 1

AVERAGE PERCENT SURVIVAL OF *Parhyale hawaiensis* IN OIL ACCOMMODATED SEAWATER

Exposure Time (Hours)	OIL CONCENTRATION						
	Control	1%	10%	20%	30%	40%	50%
24	100	100	100	100	100	100	100
48	100	100	100	100	100	100	100
72	100	100	100	100	100	100	100
96	100	100	100	100	100	100	100
120	100	100	100	100	100	100	100
144	100	100	100	100	100	100	100
168	100	100	100	100	100	95	100

TABLE 2

FIVE MOST ABUNDANT SPECIES OF ZOOPLANKTON COLLECTED AT PIER LAB OFF PORT ARANSAS

Species	Individuals Collected	Percent Total
Acartia tonsa	2421	65
Paracalanus crassirostris	986	26.3
Oithona colcarva	246	6.6
Corycaeus amazonicus	22	0.6
Eucalanus monachus	17	0.5
	TOTAL	99.0

TABLE 3

AVERAGE PERCENT SURVIVAL OF COASTAL ZOOPLANKTON IN OIL ACCOMMODATED SEAWATER

	Oil Concentration					
	Control	1%	10%	20%	30%	40%
Sample 1	47	65	82	91	96	80
Sample 2	87	88	83	81	76	83
Sample 3	67	76.5	82.5	86	86	81.5

components such as benzenes and naphthalenes must have already evaporated to the extent that the residual components are no longer able to induce any significant acute toxicity to these two marine invertebrates, amphipods and copepods. This confirmed our previous finding that weathered oil was much less toxic than fresh oil (Lee *et al.*, 1978).

Chemical analysis of the test oil also showed that only a small amount of oil is dissolved into seawater and this total water soluble fraction was less than 3 ppm (K. Winters, personal communication). The water soluble fractions of a few oils have been characterized. Anderson *et al* (1974) reported 23.75 ppm for South Louisiana crude oil, 21.65 ppm for Kuwait crude oil, and 5.28 ppm for a No. 2 fuel oil, while Winters *et al* (1976) quantified another four EXXON fuel oils; 16 ppm for Montana, 19 ppm for Baytown, 14 ppm for New Jersey, and 9 ppm for Baton Rouge.

In general, toxicity of either oil in seawater or water soluble fractions were positively related to the concentrations of aromatics such as benzenes and naphthalenes (Anderson *et al.*, 1974; Byrne and Calder, 1977) and the total amount of organics present in seawater (Lee, unpublished manuscript). Values of LC_{50} also varied with animals. For example, the 96-hr-LC_{50} of a No. 2 fuel oil was about 3.0 ppm (OAS) and 3.5 ppm (WSF) for the grass shrimp, *Palaemonetes pugio*. Under the same test conditions, the 96-h-LC_{50} for the postlarvae of the brown shrimp, *Penaeus aztecus*, was 9.4 ppm (OAS) and 4.9 ppm (WSF) respectively (Anderson *et al.*, 1974). For the two taxa, copepods and amphipods, tested in this present study, values of 96-hr-LC_{50} were above the concentrations tested (13.5 ppm). Obviously this weathered Mexican oil was far less toxic than either South Louisiana crude or some No. 2 fuel oil.

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SECTION E

MEXICAN OIL SPILL: INVERTEBRATE TOXICITY TESTS

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INTRODUCTION

On 3 June 1979, a blowout of the drilling rig IXTOC I in the Bay of Campeche resulted in the massive release of oil into the Gulf of Mexico. Immediately after the blowout the developing oil slicks began moving northeasterly in the Gulf of Mexico. The increased probability of potential damage to coastal environments along the northwestern Gulf of Mexico as the oil moved towards U.S. waters prompted the United States Coast Guard to begin planning actions of defense. One of the immediate questions faced was the potential toxicity of this Mexican oil to marine biota inhabiting probable areas of impact.

On 1 August 1979 the U. S. Coast Guard contracted the University of Texas Marine Science Institute to conduct bioassays on selected flora and fauna of the South Texas coast in order to evaluate acute toxicity effects of the Mexican oil from IXTOC I. The objective of the experiments detailed below was to quantitatively define acute effects of this oil on common invertebrates of the Texas coast that are either of direct commercial and economic importance, indirectly important to fisheries through trophic links, or are representative of fauna which may be contaminated (*i.e.* a sandy beach). We investigated effects of oil on four species of invertebrates that included the commercial brown shrimp, *Penaeus aztecus*, a sandy beach bivalve, *Donax variabilis*, an intertidal burrowing detrital feeding polychaete, *Scolelepis texana*, and a common tidal crustacean, *Emerita* sp., important in tidal marine food webs (*e.g.* shore birds). In addition to the quantitative assessment of short-term lethal effects, the experimental design also called for monitoring of changes in behavior at sublethal levels and differences in metabolic function between treated and untreated populations of the test organisms.

MATERIALS AND METHODS

The intertidal organisms were collected from the beaches of Mustang and St. Joseph Islands according to standard invertebrate collection techniques. Corresponding sediment from the organisms' habitat was collected simultaneously and sieved through a 500 μ mesh sieve to remove all other macrofauna. This sediment was dried for 48 hours before use to kill all remaining fauna. Young adult *Penaeus aztecus* (8-12 cm, total length) were obtained from a commercial fisherman whose catch came from the Corpus Christi ship channel. All organisms were acclimated in the laboratory for 48 hours (at a water temperature of 25°C) prior to the initiation of experiments. The beach fauna were maintained in salinities of 34 ppt and the shrimp in salinities of 27 ppt.

Sediment Selectivity Tests

The bottom sections of 1/2 pint capacity milk cartons, cut 6 cm from the bottom were filled with dried beach sediment that had been mixed with different concentrations of volume of whole oil. The range of concentrations included controls (no oil in sediment), 0.1%, 1.0% and 10.0% oil in sediment. Cartons containing control, 0.1% and 1.0% oiled sediment were placed randomly side by side in a 20-gallon aquarium. A separate 10-gallon aquarium was used for control and 10.0% oiled sediment cartons in case the higher oil concentrations might interfere with the interpretation of results for the 0.1% and 1.0% oiled sediments. The aquaria were slowly filled with beach water (34 ppt salinity) to a depth of 12 cm over the surface of the sediments. Mole crabs (*Emerita* sp.) were introduced randomly over the water surface. The water was aerated and maintained at 25°C. One experiment was run for 24 hours and a second was run for 72 hours. At termination of the tests, the water was slowly drained to below the

surface of the sediment containers. The contents of each carton were sieved through a 500 μ mesh sieve and the living and dead mole crabs counted immediately.

Toxicity Bioassays

Oil accomodated water (OAW) was obtained from the chemical group of the Marine Laboratory (see chemical report for preparation methods and oil concentration). This stock solution, which contained approximately 25 ppm total hydrocarbons, was used to prepare exposure solutions for each of the bioassay tests. Calculated treatment exposure concentrations of OAW for each of the test animals were 0 (control), 5%, 15%, 30% and 60% in seawater. Twenty-four (24) hour water samples taken for hydrocarbon analysis indicated a significant evaporation of the hydrocarbons. Therefore, each test container for the mole crabs, surf clams and polychaetes had 25% of its water removed and replaced with a fresh mixture of exposure solution daily. The treatment tanks for the shrimp were replaced with a fresh OAW mixture at the end of 48 hours.

Mole Crab (*Emerita* sp.)

Four groups (replicates) of crabs consisting of 10 animals/group were exposed to each treatment concentration. These animals were placed in 11.4 cm finger bowls containing approximately 1.5 cm of sediment and 200 ml of 34 ppt seawater which was aerated. The animals were fed rotifers (*Brachionus plicatilis*) daily. Each bowl was censused every day and deaths recorded. In addition to mortality observations, these animals were observed for behavioral differences. Before feeding, crabs were observed for their position in relation to the surface of the sediment (exposed or buried). There were two major types of activities noted: 1) the anterior portion of the body was exposed with no noticeable

feeding activity; and 2) anterior portion of the body exposed and second antenna actively filtering water, indicative of feeding. After feeding, the above observations were repeated to note any differences due to food stimulation. The experiments were terminated after 96 hours. Remaining live mole crabs and pieces of carapace of dead and/or molted crabs were retrieved. Percent mortality and survival were calculated for each treatment. Percent survival was determined as the percentage of live mole crabs remaining in the bowls at the end of 96 hours.

Surf Clams (*Donax variabilis*)

Four groups (replicates) of clams consisting of 10 animals/group were exposed to each treatment concentration for 96 hours. These animals were placed in 11.4 cm finger bowls containing approximately 3 cm of sediment and 200 ml of 34 ppt aerated seawater. The animals were fed daily with rotifers. Each bowl was censused every day for deaths. Behavioral differences between treatments were also recorded daily. Clams were observed to record the working of their siphons, *i.e.* the occurrence of water current exchange. The following observations were recorded after feeding: 1) the number of clams with siphons extended and working; 2) the number of clams with siphons continually extended for a duration of 2-3 minutes; and 3) number of clams with no obvious activity during the observation period. The experiments were terminated after 96 hours and the percent total mortality for the surf clam in each treatment was calculated.

Polychaete (*Scolelepis texana*)

Four groups (replicates) of polychaetes consisting of 10 animals/group were exposed to each treatment concentration. These animals

were placed in 11.4 cm finger bowls containing approximately 1.5 cm of sediment and 200 ml of 34 ppt aerated seawater. Animals were fed rotifers daily. Each bowl was censused at the end of 24 hours for a 96 hour period. Dead worms were recorded and removed from the bowls. Dead and decomposed posterior and anterior (branchial) body sections of worms were also noted and removed from the dishes. These, in addition to the intact dead worms, were later used to determine mortality within each bowl over the 96-hr period. In addition to mortality observations, the worms were observed for behavioral differences. Each bowl was observed daily under a dissecting scope to determine 1) percent activity, the number of the total worms for each treatment which were extended from a burrow opening and moving about on the surface of the sediment; 2) the number of burrow openings per bowl on the surface of the sediment and, of these, the number with fecal pellets around the openings. The above observations were made 30 minutes after feeding. The experiments were terminated after 96 hours, remaining live worms and segments of dead worms retrieved from the bowls, and percent mortality and percent survival calculated for each treatment. Percent survival was determined as the percentage of live polychaetes remaining in the bowls at the end of 96 hours.

Brown Shrimp (*Penaeus aztecus*)

Four groups (replicates) of shrimp consisting of eight animals/group were exposed to each treatment concentration. These animals were placed in aquaria containing approximately 4 cm of sediment in 14.5 liters of 27 ppt aerated seawater. Animals were not fed. The tanks were sealed with black polyurethane plastic to simulate darkness. Each tank was censused at the end of 24 hours for a 96 hour period. Dead shrimp

were recorded and removed from the tanks. Shrimp activity was monitored daily by subjecting each tank to daylight for 30 minutes to stimulate burrowing activity. After 96 hours the shrimp were exposed to light for 5 minutes and percent burrowing was recorded. The experiments were then terminated.

Oxygen Consumption

Differences in metabolic function of control and treated populations of test organisms were determined by oxygen consumption. The following tests were run:

Organism	No. of Replicates	No. of Organisms/ Replicate	Duration of Measurement	Treatments Tested
Mole Crab	3	3	30 min.	Control,60% OAW
Surf Clam	3	3	30 min.	Control,60% OAW
Polychaete	2	10	30 min.	Control,60% OAW
Shrimp	3	1	20 min.	Control,15 & 60% OAW

All organisms were acclimated to 25°C for 15 minutes. Respiration chambers were filled with supersaturated filtered seawater and held to a constant temperature (25°C) in a circulating water bath. A Beckman model 0260 oxygen analyzer ($O_2/T°$) was used and changes in oxygen (ppm) within the respiration chamber were recorded every five minutes for the duration of the measurements. Organisms were wet weighed to the nearest .001 g. Oxygen consumption was calculated for each replicate in ppm O_2 hr^{-1} g^{-1}.

RESULTS

The results of the 24 hour sediment selectivity tests suggested that the mole crab had a definite preference for the less oiled sediments (Figure 1). There was a significant difference between treatments at $P < 0.01$

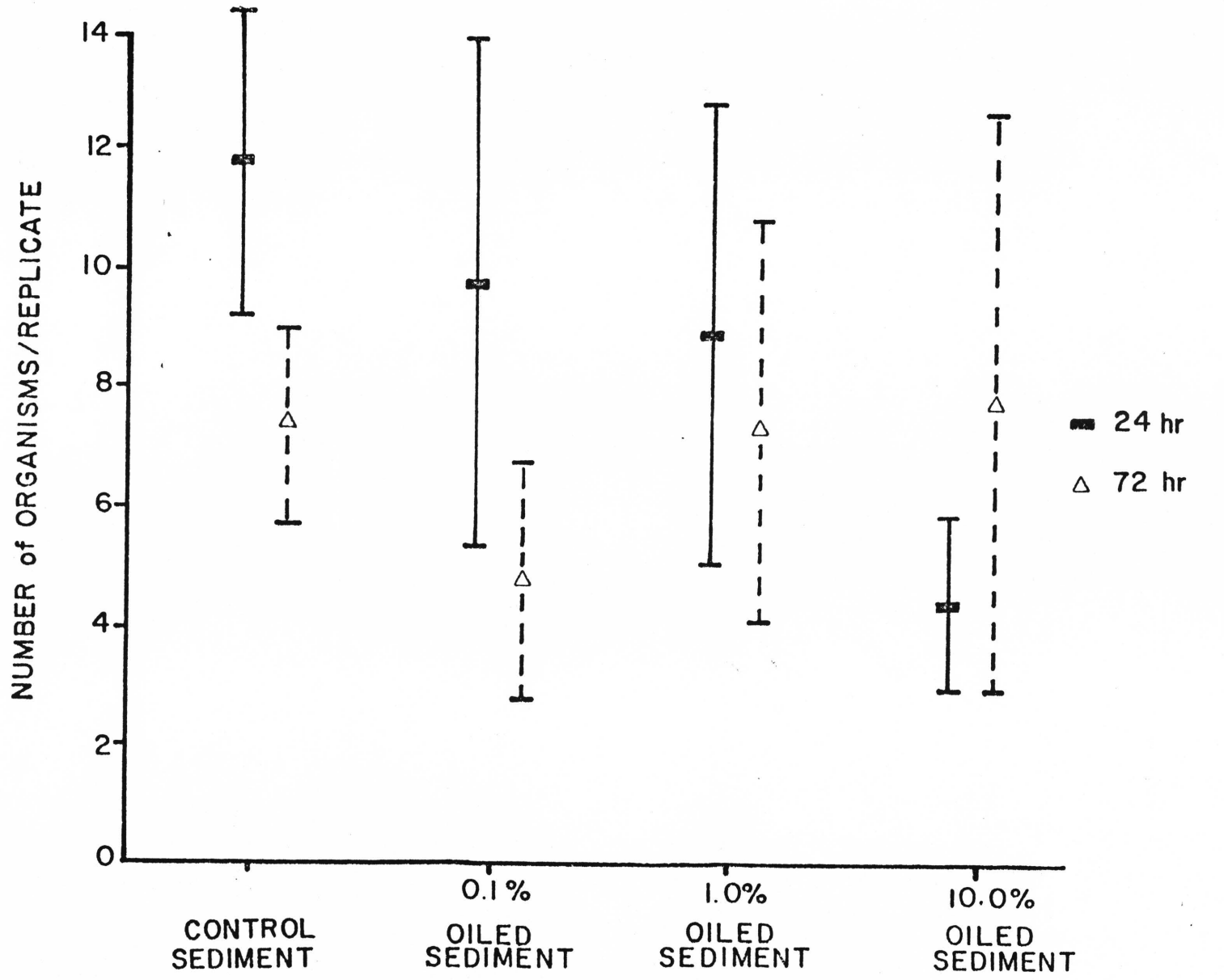

Figure 1. Results of sediment selectivity tests with *Emerita* sp. as the test organism. Two tests were run, one of 24 hr duration and the second of 72 hr duration.

as tested by one-way ANOVA for these 24 hour tests. Thirty-five (35) percent of the crabs were observed in the control sediments, 28% in the 0.1% oiled sediments, 25% in the 1.0% oiled sediments, and 13% in the 10.0% oiled sediments. Less than one percent mortality was observed in all treatments over the experiment duration and no treatment showed significantly greater mortality than the controls.

The 72 hour sediment selectivity tests showed much different results. For these tests there were no significant differences in selecting any of the treated sediments by the mole crabs at $P < 0.05$ (Figure 1). This experiment did show a slightly different result concerning crab mortality. No dead crabs were observed in the control sediment. Mortality was 1% in the 0.1% oiled sediments, and 2% in the 1.0% and 10.0% oiled sediments.

Toxicity Bioassays

Mole Crab

Percent mortality, percent survival (as described in methods), and percent activity (number of mole crabs exposed above the sediment following food stimulation) are recorded below:

Treatment	% Mortality				% Survival	% Activity # Visible after Food Stimulation			
	24-hr	48-hr	72-hr	96-hr	96-hr	24-hr	48-hr	72-hr	96-hr
Control	0	0	0	0	95	35	63	40	18
5% OAW	0	0	0	0	98	60	80	50	33
15% OAW	0	0	0	0	98	68	70	50	33
30% OAW	0	0	0	0	75	63	58	60	25
60% OAW	0	0	0	0	100	78	70	53	38

No mortalities of mole crabs were recorded for any of the OAW treatments. Not all of the tests organisms were retrieved from the experiments. These unretrieved animals as well as pieces of mole crabs and molts may represent

mortalities which could not be verified with an in-hand dead specimen. Acute sublethal effects as determined by mole crab activity measurements were not noticeably different for the treatments. Percent activity decreased throughout the 96-hr period.

Surf Clam

Percent mortality and percent activity (number of surf clams with siphons extended and working following food stimulation) are recorded below:

Treatment	% Mortality				% Activity # w/siphons extended & working			
	24-hr	48-hr	72-hr	96-hr	24-hr	48-hr	72-hr	96-hr
Control	0	0	0	8	40	33	30	5
5% OAW	0	0	0	0	53	38	28	25
15% OAW	0	0	0	0	68	48	40	28
30% OAW	0	0	0	0	58	23	40	20
60% OAW	0	0	0	0	38	28	35	18

No mortalities were recorded for any of the surf clams in the OAW treatments (8% in the control). All test animals were retrieved from the experiments. Sublethal effects as determined by surf clam activity measurements were not noticeably different for the treatments. Percent activity decreased throughout the 96-hr period.

Polychaete

Percent mortality and survival (as described in methods) are recorded below:

Treatment	% Mortality				% Survival 96-hr
	24-hr	28-hr	72-hr	96-hr	
Control	0	3	5	10	85
5% OAW	3	5	5	8	80
15% OAW	3	10	10	10	75
30% OAW	5	5	8	8	92
60% OAW	10	17	17	23	77

Not all the test organisms were retrieved from the experiments. These

unretrieved animals as well as dead and decomposing body sections may represent mortalities which could not be verified with an in-hand dead specimen. The highest mortality (23% at 96 hours) occurred in the 60% OAW treatment. Sublethal effects as determined by polychaete activity could not be determined for any of the treatments. The number of polychaetes which were extended from their burrows after 24 hours was minimal for all treatments. Burrow openings and fecal pellets around openings were recorded, but artifacts of the previous day's activity could not be distinguished from the current day's. In general, activity increased in all treatments after 48 hours then decreased through 96 hrs below the initial level.

Qualitative observation of polychaetes after retrieval from experiments and prior to and during respiration measurements showed the 60% OAW organisms to be in poor condition, alive but motionless upon stimulation, and covered with mucus and attached sand grains and debris. The polychaetes from the control dishes, however, were robust, active, in good condition, and free of mucus and debris.

Shrimp

Percent mortality for the shrimp at the various treatment levels are recorded below:

	% Mortality			
Treatment	24-hr	48-hr	72-hr	96-hr
Control	3	6	6	6
5% OAW	3	3	3	3
15% OAW	0	0	0	0
30% OAW	3	3	3	3
60% OAW	9	9	9	9

All test animals were retrieved from the experiments. The highest mortalities (9% at 24 to 96-hr) occurred in the 60% OAW treatment.

Sublethal effects as determined by shrimp burrowing activity for 30-min of light exposure were not quantifiable. Initially most shrimp would burrow. Then within the 30-min period, others would come out of the sediment and burrow back up or remain on the surface of the sediment. After 96-hrs the shrimp were exposed to light for 5-min. and percent burrowing was recorded. None of the burrowed shrimp resurfaced during this time. This method was a more accurate representation of activity for the shrimp and the results are recorded below:

Treatment	% Burrowed Initially	% Burrowing w/in 5 min.	% Burrowing at end of 5 mins.
Control	41	42	75
5% OAW	31	59	59
15% OAW	47	59	69
30% OAW	77	43	90
60% OAW	71	50	93

In most treatments, approximately 50% of those above the sediment would burrow within the 5-min. exposure to light. Number of burrowed shrimp was highest in the 30 to 60% OAW treatments, both before and after exposure to light (5 mins.).

Oxygen Consumption

Oxygen consumption (ppm O_2 hr^{-1} g^{-1}) for organisms taken from various oiled treatments after 96-hrs are recorded in Table 1. The only measurable differences that were shown to be significant ($P < 0.05$) between the control and any oiled treatment were for the mole crab. There appeared to be an increase in oxygen uptake in the oiled vs. the control treatments for the surf clam and shrimp but these differences were not significant. Oxygen consumption was lower for the polychaetes treated with 60% OAW than for the control and may possibly be a refelction of the poor physical condition of these worms as noted in the toxicity results section.

Table 1. Oxygen consumption for experimental animals removed from control, 15% and 60% OAW treatments after 96 hours. Data are reported in ppm O_2 hr^{-1} g^{-1}.

Organism		TREATMENT		
		Control	15% OAW	60% OAW
Mole Crab*		20.14		18.24
		24.88		18.47
		26.95		15.32
	$\bar{X}$	23.99		17.34
Surf Clam		6.44		9.80
		10.12		13.36
		11.39		15.18
	$\bar{X}$	9.32		12.78
Polychaete		90.00		85.37
		142.86		90.90
	$\bar{X}$	116.43		88.14
Shrimp		1.62	1.67	1.29
		1.05	1.84	1.39
		1.35	1.32	1.68
	$\bar{X}$	1.34	1.61	1.45

*Significant difference at $P < 0.05$

DISCUSSION

The complexity of assessing the impact of petroleum hydrocarbons on the marine biota investigated here was well documented by the results of these tests. Few observable impacts were noted and a problem exists in extrapolating these results to the real ecosystem.

We did not determine lethal doses of the IXTOC I oil accomodated water to the biota studied. This could be related to the physical nature of the oil tested. It is also possible that the solutions tested (*i.e.* 5 to 60% of 25 ppm) did not mimic actual concentrations that are presently occurring in the Gulf of Mexico and on Texas beaches.

Although by no means significant, we did observe a greater amount of mortality in the polychaetes than in any other fauna. This may be related to their feeding methods. The polychaete was the only one of the three beach fauna tested that was a surface deposit feeder. The other two were both suspension feeders. Feeding from the sediment surface, the polychaete may have injested the numerous micro-tarballs that were suspended in the OAW at treatment initiation and later settled to the sediment surface. This may help to explain the poor health of the worms in higher treatment concentrations as noted in the results.

These tests also did not evaluate any of the sublethal biological effects on reproductive success, long-term health of the organisms, accumulation of toxic substances, growth and development, or histopathological conditions. Many of these biological aspects may have been affected from chronic exposure to the Mexican oil. All that can be concluded here is that at the concentrations tested, the fauna examined appeared to show very few acute effects from oil treatment.

Although these tests were not conclusive in terms of any significant

mortality effects from acute exposure to the Mexican oil tested, because of the numerous unknowns including some of the questions raised above, we urge that care be taken to protect the south Texas marine environment from potential unknown effects related to repeated exposure.

SECTION F

EFFECTS OF IXTOC I GULF OF MEXICO OIL SPILL MATERIALS ON THE BEHAVIOR AND RESPIRATORY METABOLISM OF THE SPOTTED SEATROUT (*Cynoscion nebulosus*)

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ABSTRACT

Exploratory experiments with a seawater extract (30 mg l^{-1} oil) of IXTOC I crude oil "mousse" were behaviorly deleterious to subadult spotted seatrout (Cynoscion nebulosus) at about 7.5 mg l^{-1} or higher immediately and after 24 hrs at 0.3 mg l^{-1} or higher. A similar experiment with fingerlings indicated a 96-hr TLM level in the range of 1.3 - 7.5 mg l^{-1}, with about 10 g or smaller fish the more sensitive.

Blazka respirometer experiments at near ambient temperatures of 28°C and at 35 o/oo salinity to measure metabolic levels up to maximum sustained swimming velocities indicated lethality within about two days at 5% dilutions of the seawater extract. Similar Blazka respirometry indicated that 1% dilutions would be effective up to about 4 days without perceptible morbidity.

At 1% average maximum sustained swimming velocities were reduced 85% to 2.8 lengths sec^{-1} from 3.3 L sec^{-1} for the controls. From 2 to 7 days the performance levels decreased, but not from 2 to 5 days for the controls. During these periods the usual stress symptoms of behavioral and physical deterioration appeared after about four days at the 1% concentration, but not for the controls.

Multiple regressions relating oxygen consumption rate to the body weight and swimming velocity made comparisons possible between control and experimental fish.

At the average weights of the fish in each of the regressions the oxygen consumption rates were calculated for the active rates at average maximum sustained swimming velocities and at standard, zero

velocity, rates. The oxygen consumption rates as metabolic rates per kilogram yielded metabolic scope values as the difference between active and standard rates. The pollutant reduced the scope to at least 89% of the control values, even though the "standard" was reduced from that of the controls.

For larger fish (500 g) the same calculations of active and standard rates from the regressions yielded a scope reduction to 67% of controls. Thus it appears that larger fish, along with very small fish, are progressively more sensitive to the toxic materials.

The relationships of the regression constants and statistics indicate that metabolic responses of the spotted seatrout to the toxicants are generally more variable than to control conditions.

These preliminary experiments suggest that the Blazka respiratory metabolism technique of determining metabolic scope and its reduction under sublethal stresses is highly sensitive and quantitative. It can be used when there is no a priori chemical information on the nature and effects of toxic materials.

INTRODUCTION

This study was designed to contribute preliminary information on toxicity levels and sublethal respiratory metabolic effects of Bay of Campeche crude oil on Gulf of Mexico fish species. The spotted seatrout (Cynoscion nebulosus) was the fish of choice due to its occurrence in both nearshore and estuarine environments. It is a sensitive, euryplastic species on which a considerable amount of baseline data have been acquired in studies by Wohlschlag and Wakeman (1978) and Wohlschlag and Parker (in progress).

Initial toxicity tests were conducted on adult C. nebulosus at various concentrations of oil accommodated in water (OAW) prepared from samples of "mousse" collected by the U.S. Coast Guard Cutter Point Baker. A second set of toxicity tests were made utilizing fingerling or underyearling trout to ascertain size-related effects. Rationale for these experiments included determination of toxic concentrations of the spill material and establishment of appropriate concentration levels for investigation of sublethal effects on the respiratory metabolism and swimming performance of C. nebulosus.

Respiratory metabolic responses of marine fish have been shown sufficiently sensitive to sublethal pollutant levels to be useful in biological monitoring (Wohlschlag et al. 1978; Wohlschlag and Parker 1978). The use of respiratory metabolism is based on the fact that it is often measurably influenced by toxic substances in dilutions far below usually perceived lethal concentrations. The use of respiratory scope -- the difference between oxygen consumption at the maximal sustained aerobic activity and at the

minimal maintenance, or standard, level -- for the assessment of environmental quality was suggested by Fry (1947, 1957, 1971). Theoretical and empirical studies indicate that metabolic scope tends to be reduced by stresses when standard rates may be increased, active rates reduced, or both. Wohlschlag et al. (1978) and Wohlschlag and Parker (1978) have demonstrated scope reductions in the red snapper (Lutjanus campechanus) exposed to sublethal concentrations of ocean dumped industrial wastes.

Ecologically, most fishes generally operate at a routine rate that lies between the standard and maximum. This rate is operationally minimal and is around twice the standard level to account for about 1 length sec^{-1} swimming (foraging), specific dynamic action (assimilation) and other functions, excluding growth, spawning, extended migrations, etc. (Fry, 1971; Kerr, 1971; Mann, 1969; Winberg, 1956; Wohlschlag and Wakeman, 1978). Stresses also can depress routine metabolic rates (Beamish, 1964; Wohlschlag and Cameron, 1967; Kloth and Wohlschlag, 1972; Cech and Wohlschlag, 1975), although a depressed routine rate appears to be less definite than scope for maximal sustained activity for species that may have maximal swimming metabolic activity levels 4 - 8 times standard levels (Randall, 1970).

The specific aims of these studies were to use the spotted seatrout, a well known commercial and recreational species, as a test organism to make preliminary determinations:

1. On acutely toxic levels of oil spill materials on adults and fingerlings or underyearlings;

2. On metabolic results at active and standard (or resting) levels for detection of scope diminution even though the chemical composition of the spill material could be considered unknown;

3. On what levels of the spill material produce observable metabolic depression;

4. For additional information of basic energetics data on a species of general importance in fishery and ecological considerations.

MATERIALS AND METHODS

Spotted seatrout were used throughout the study and were captured near Port Aransas, Texas by hook-and-line or seine. Capture temperature (30C) and salinity (32 o/oo) were near test levels for respiration experiments but toxicity test fish required additional acclimation to the lower (23C) room temperature used. Fish were transported to the laboratory in insulated boxes and placed in indoor holding tanks for acclimation to the appropriate test condition. Any fish that behaved abnormally or appeared unhealthy were discarded.

The preparation of the oil spill material to produce a stock "100% solution" was carried out as follows: Point Baker "mousse" was blended with seawater from laboratory settling tanks at 35 o/oo. No measured amounts of "mousse" were used; the procedure involved high speed blending of the oil in water for 45 seconds, after which the water was allowed to clear for a few seconds, then the water was siphoned into a holding container. This water fraction will be referred to as oil accommodated in water (OAW). After a sufficient quantity of this solution was collected it was diluted with two parts seawater to produce what we will call a 100% solution. Prior to the onset of each test and at the termination point, samples of each appropriate dilution were taken for later chemical

analysis. Initially the concentration of the stock was 27 mg l^{-1} oil for the adult fish toxicity tests and 30.1 mg l^{-1} for the fingerlings and underyearlings.

Analyses have been made by Dr. Kenneth Winters and further detailed chemical analyses are pending.

Toxicity tests on adult C. nebulosus were conducted in 20-gallon glass aquaria equipped with aerators. Six dilutions were used (100%, 50%, 25%, 12.5%, 5% and 1%); control fish were maintained in a larger holding tank. Observations were recorded for a 96-h period on each of the 6 tanks which contained 2 fish of 100 - 200 g weight. No temperature control was available for toxicity tests, so that room conditions dictated 23C and a salinity of 35 o/oo was used throughout all tests.

Fingerling or underyearling fish tests were carried out in 6 20-gallon aquaria at 5 dilutions (100%, 50%, 25%, 12.5%, and 5%) and one control tank. Again observations on behavior and any fatalities were recorded for a 96-h period. At the termination of all experiments, fish were weighed and lengths measured. Specimens were then frozen for later inspection.

From the behavioral observations on the adult fish it was determined that respiration experiments should be attempted at 5% of the 100% dilution. However this dilution produced 100% mortality in 63.5 hours at 28C and 35 o/oo during a 2-day+ acclimation period. The test dilution was then lowered to 1%. Fish were held in a temperature controlled circular tank at 1% OAW dilution for 2 days prior to testing. Fish were fasted during

this period and a slow current within the circular tank provided some acclimation to swimming before respiration measurements. The holding tank and respirometer tank were well aerated throughout experiments.

Active and resting metabolism rates were made in a 207-l Blazka chamber (Blazka et al., 1960; Fry, 1971) as utilized by Wohlschlag and Wakeman (1978). The entire chamber was immersed in a 3,024-l temperature-salinity controlled system, which was a contiguous part of the circular holding tank. No filtration system was utilized for these tests. Fish were maintained for 2 days swimming at low velocities (about 1 L sec^{-1}) prior to active measurements. After swimming in the chamber at an intermediate speed for a period of time sufficient to calm the fish, the velocity was increased gradually until the fish "broke" pace. At this instant the velocity was lowered (usually quite slightly) to the highest possible velocity at which normal swimming persisted without breaking. With this "training" regimen, the maximum sustained velocity could be reproducible for each fish. The U_{max} (total lengths sec^{-1}) swimming velocity was determined, after which the fish was tested for at least 1 h for a consistent U_{max}. Following the 1 h or longer runs, the fish were left in the chamber at intermediate and/or zero velocities with oxygen rate measurements to detect any respiratory irregularities that could have resulted had the U_{max} been associated with undesirable anaerobic metabolism.

Oxygen consumption rates were measured by withdrawal of small samples for use in a Radiometer model E-5046 with a PHM 71 electrode equipped with acid-base analyzer. Following completion of a set of

experimental oxygen consumption measurements, the fish were removed and lengths and weights recorded. Along with lengths, weights, oxygen consumption rates, and swimming velocities (total lengths sec^{-1}), salinities and temperatures were recorded to 0.1 ppt and 0.1C. From this, a simple multiple regression was calculated at control and experimental conditions in the form:

$$\hat{Y} = a + b_w X_w + b_v X_v$$

where:

$\hat{Y}$ = expected O_2 consumption rate in $\log_{10}$ mgO_2h^{-1},

a = constant,

X_w = $\log_{10}$ weight in grams,

X_v = 1 sec^{-1}.

The various $\underline{b}$ values are the respective partial regression coefficients. Similar procedures have been used by Wohlschlag and Juliano (1959), Wohlschlag and Cameron (1967), Wohlschlag and Cech (1970), and others.

Temperature and salinity values remained near 28C and 35 ppt respectively and were not included in the regression calculations. Control data were acquired from a study on ocean dumped pharmaceutical wastes by Wohlschlag and Parker (in progress). Standard metabolic rate determinations were made from the appropriate active regression equation utilizing the Brett (1964) technique. This involves drawing a line parallel to the active regression line through the lowest U_{max} value and using the Y intercept value as a realistic estimate of the standard rate.

RESULTS

The preliminary experiment to assess the toxicity of the oil accommodated water (OAW) to subadult fish in 20-gallon aquaria indicated that 100%, 50% and 25% dilutions were definitely deleterious -- at least behaviorly over 96 hours. At dilutions of 12.5%, 5% and 1% adverse effects usually appeared about 24 h later. The descriptive details are in Appendix Table 1.

The second preliminary experiment to assess 96 h toxicity to fingerlings with similar but more definitely lethal results is described in Appendix Tables 2 and 2a. From the crude results a 96 h TLM level would be a range from 5-25% dilution (1.3 - 7.5 mg l^{-1}). The smallest of the fish generally died first; that would indicate a critically susceptible size smaller than about 10 g, although there may have been uncontrolled variables from one aquarium to the next.

The first Blazka respiratory measurement attempts under controlled conditions failed during acclimation (habituation) to a 5% OAW level when some deaths occurred. In the appendix (following Table 2a) are appropriate notes that indicate severity of the stress at 5% OAW. Accordingly the remainder of the results deal with respiratory metabolism measured under control or 1% OAW conditions in the Blazka apparatus.

The more definitive results from the Blazka chamber experiments yield the following equation for control data:

$$\hat{Y} = 0.06995 + 0.71242\ X_W + 0.11192\ X_V, \quad \text{(Eq. 1)}$$

where the average weight was 114 g and the average of 14 determinations at U_{max} was 3.3 L sec^{-1}. Total N = 34.

The data at 1% OAW yield:

$\hat{Y} = 0.87662 + 0.36468\ X_w + 0.11381\ X_v$, (Eq. 2)

where average weight was 164 g, average of 10 U_{max} measurements was 2.8 L sec^{-1}. Total N = 27.

The following schedule of statistics for these equations are also useful:

Eq.	N	R	s_y	s_{b_w}	P	s_{b_v}	P
1	34	0.92	0.07901	0.12416	<0.001	0.0092	<0.001
2	27	0.77	0.13624	0.02123	<0.001	0.0190	<0.001

Fig. 1 is the plot of oxygen consumption rate of all control fish (calculated from Eq. 1 and adjusted to the average weight of 114 g) against observed swimming velocities. The equation is

$\hat{Y} = 1.53533 + 0.11192\ X_v$.

The Brett (1964) extrapolated standard metabolism rate is 29.17 mg $O_2 h^{-1}$ or 256 mg $O_2 kg^{-1} h^{-1}$.

Fig. 2 is the plot of the oxygen consumption of the experimental fish (calculated from Eq. 2 and adjusted to the average weight of 164 g) against observed swimming rates. The equation is

$\hat{Y} = 1.68433 + 0.11381\ X_v$.

By the Brett (1964) method, the extrapolated standard rate is 35.08 mg $O_2 h^{-1}$ or 214 mg $kg^{-1} h^{-1}$.

Some pertinent notes on the condition of the fish in the Blazka chamber experiments are most revealing. For 2 days initial acclimation and 7 more days for making the metabolic performance

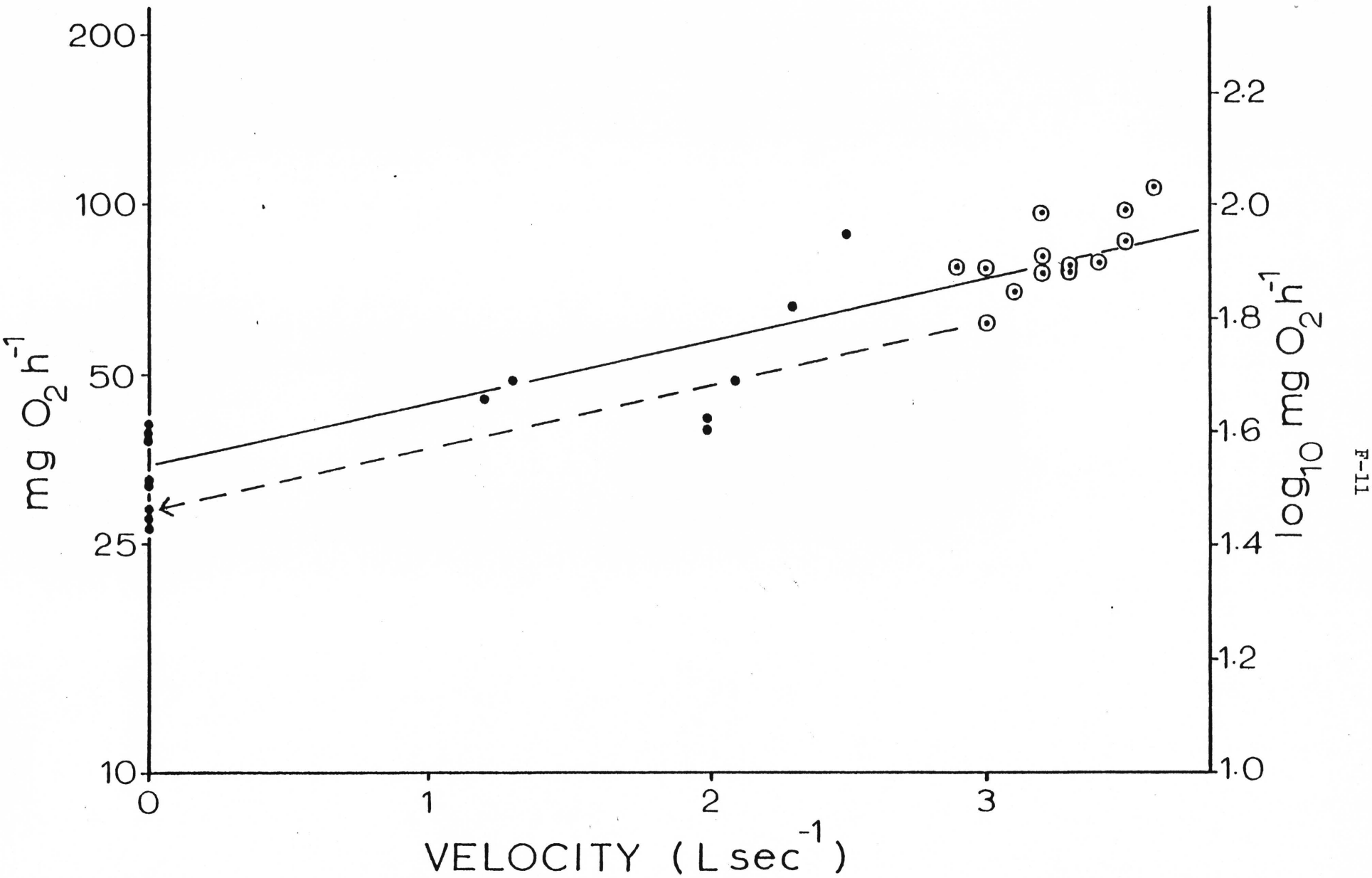

Fig. 1. Calculated oxygen consumption rates for control spotted seatrout at 114 g average weight plotted against observed swimming rates. Estimated standard rate shown by arrow (see text). Encircled points are for maximum sustained (U_{max}) swimming performances.

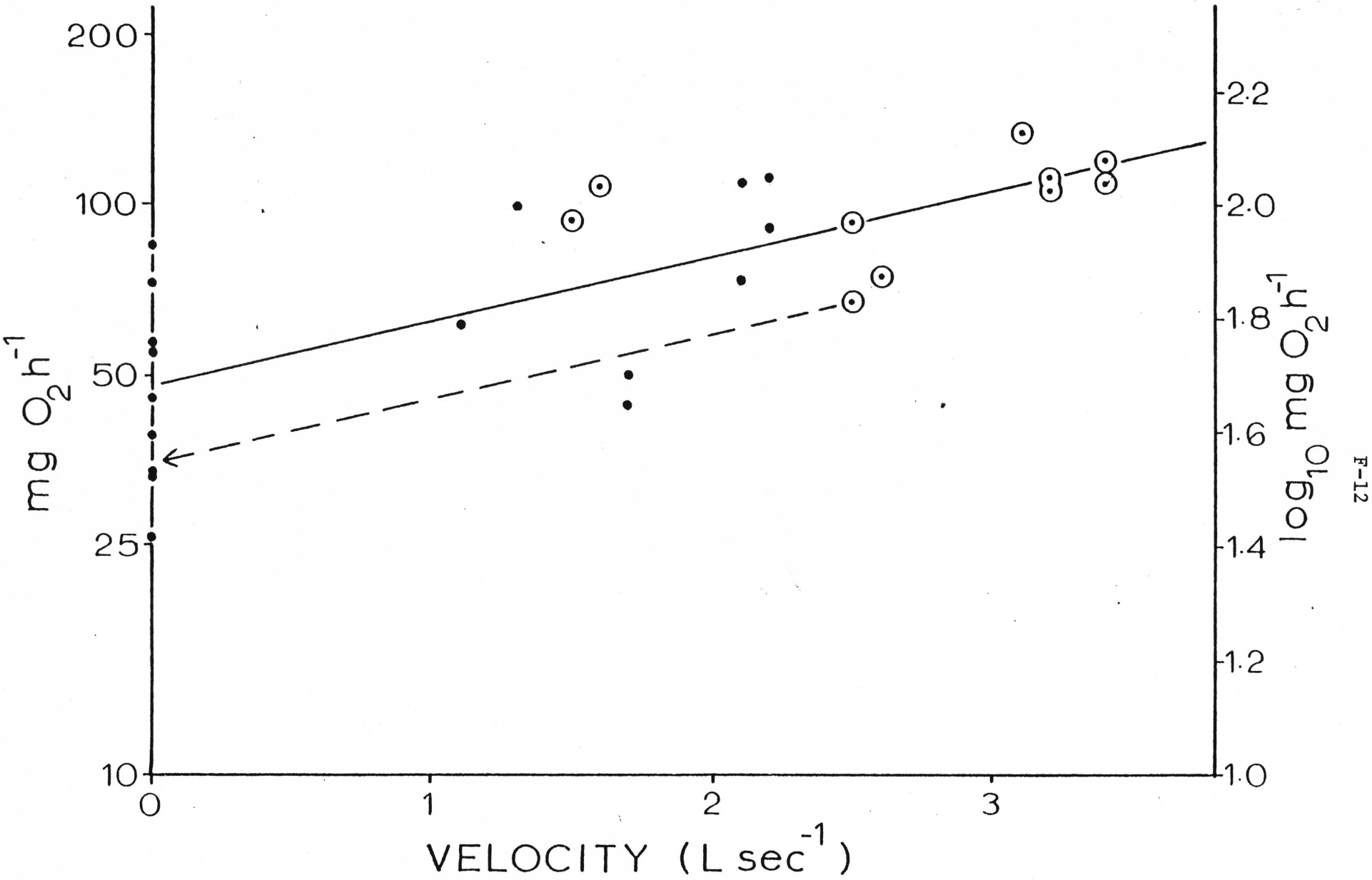

Fig. 2. Calculated oxygen consumption rates for spotted seatrout in 1% crude oil extract at 164 g average weight plotted against observed swimming rates. Standard rate estimate shown by arrow (see text). Encircled points are for maximum sustained (U_{max}) swimming performances.

runs, there was no perceptible deterioration with U_{max} values showing no trend downward. The fish in the 1% solution showed no deterioration for the first 4 days, during which no abnormal effects were observed during the 2-day acclimation period and the first 2 days of experiments. By the fifth day, fish in the Blazka chamber swam less well and appeared to have equilibrium problems. Fish were definitely sluggish in the sixth day with obvious prevalence of tail rot. Of three fish remaining on day 7 one died, one failed to swim faster than 1.6 L sec^{-1} and was afflicted with tail rot, gill lesions and coughing spasms, and the last fish with the same afflictions swam weakly at only 1.5 L sec^{-1}.

DISCUSSION

General Remarks

The initial trials which indicated a 1% OAW extract was adequately toxic, were apparently misleading, inasmuch as cumulative effects did not become evident until after 4 days. Just what protocol should be used for evaluating delay reactions to unknown toxins at unknown concentrations is not clear at this time when relative concentrations, exposure time and effects in the open ocean are unknown. Some crude oil spill pertinence to fishes is available from the literature, however, even though marine sublethal effects on fishes may be unknown in quantitative terms. Also very little laboratory information is available for larger fish.

McKeown and March (1978) have observed severe damage to gills in rainbow trout (Salmo gairdneri) exposed to Bunker C oil.

Minchew and Yarbrough (1977) found fin erosion in Mugil cephalus exposed to 4-5 mg l^{-1} crude oil in estuarine pond ecosystems. Sindermann (1978) reviewed the recent literature on the generalized nature of fin rot occurrences in degraded estuarine and coastal systems. The coughing response has been shown to be directly related to concentrations of toxic substances (Barnett and Toews, 1978; Carlson and Drummond, 1978; among others). Any of these, or other similar deficiencies, would be expected to suppress metabolic scope.

Choice of Experimental Concentration of Seawater Extract

From the descriptions of the preliminary toxicity tests and the Blazka chamber respiratory experiments, there is no obvious biological clue as to a "good" concentration or a "good" exposure interval. Whatever the identifications of toxic materials are, it is apparent that a 5% extract is acutely toxic within about 2 days. In about 7 days the 1% dilution could be considered acutely toxic. Whether the acute toxicity depends upon bioaccumulation past a threshhold concentration, or the progressive breakdown of a biochemical system once a toxin or combination of toxin initiates a degradational process, should provide an important point of departure for future experiments. Such experiments should provide contrasting acute and sublethal chronic toxicity levels with the sublethal levels (1) low enough to allow for flow-through experiments conducted at a continuously added, constant pollutant level and (2) low enough to detect further degradation, if any, by a single initial addition of the pollutant.

The experimental concentration of the pollutants in this study yield about what might be ordinarily expected of sublethal levels. The Blazka respirometer experiments as summarized by Equations 1 and 2 and the plotted processed data in Figures 1 and 2 show that the variability of the controls is considerably less with high R multiple correlation and low standard error of the regression, s_v. The greater variability of the plotted data in Fig. 2 show up at $X_v = 0$ and at the maximum sustained (encircled) values as compared to the Fig. 1 control data. The greater variability of the standard error (s_{b_w}) for the b_w of the control (0.12 compared to 0.02) is not clear, although similar ranges of standard errors are common and may be associated with the relatively smaller average size $\bar{X}_w = 114$ g and the smaller range of weights for the control fish.

Relative Scope Depression at Observed Average Weights

Average weights of 114 g for controls in Equation 1 and 164 g for 1% Ixtoc I samples in Equation 2 reveal somewhat emphatically the differences between control and experimental metabolic levels when the average maximum activity is decreased from 3.3 L sec^{-1} to 2.8 L sec^{-1}, or to 85% of the maximum sustained value.

At 114 grams, Eq. 1 yields 704 mg O_2 $kg^{-1}h^{-1}$ at maximum activity, $X_v = 3.3$ L sec^{-1}. From Fig. 1, extrapolated from the minimum active level of this zero (standard) level by the Brett (1964) method, the standard rate is 256 mg O_2 $kg^{-1}h^{-1}$. The

difference, or scope, is 448 mg O_2 $kg^{-1}h^{-1}$ (704-256).

At 164 g, Eq. 2 (1% Ixtoc I) yields at X_v = 2.8, 614 mg O_2 $kg^{-1}h^{-1}$, which is considerably depressed. A corresponding Brett type of extrapolation gives a standard rate of 214 mg O_2 $kg^{-1}h^{-1}$, somewhat lower than the control as might be expected for continuously stressed fish. The difference, 614-214, 400 mg O_2 $kg^{-1}h^{-1}$ is a scope that is about 89% of the 448 control values.

The depression in the b_w coefficient in Eq. 2 to 0.36 compared to 0.71 in Eq. 1 indicates that polluted waters adversely and selectively affect the larger fish. This situation has been repeatedly observed both in current studies in progress on effects of industrial wastes and in published studies by Wohlschlag and Cameron (1967), Kloth and Wohlschlag (1972), among others. For this study an extrapolation from Equations 1 and 2 to a larger size, say 500 g, is instructive. For control fish the scope is (460 - 167) = 293 mg O_2 $kg^{-1}h^{-1}$ with X_w = 500 g and X_v = 3.3 L sec^{-1} in Eq. 1. For the oil exposed fish, the scope is (302 - 105) = 197 mg O_2 $kg^{-1}h^{-1}$ at X_w = 500 g and X_v = 2.8 L sec^{-1}. Thus for a 500 g fish the oil exposed fish have a scope value that is about 67% of the control fish.

Clearly the implications are that even these low levels of chemical toxicants in the 1% solution of the mousse water phase can have a severe effect on the overall metabolism of organisms. In fisheries the disappearance of larger members with exploitive stresses is well known, but little work has been extended to show how natural or pollution stresses at very low, sublethal levels

can have the same ultimate effect, i.e. the older and larger members tend to disappear from a population structure while the younger and smaller members survive -- providing some recruitment is maintained, of course.

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APPENDIX

TABLES AND NOTES

Appendix Table 1

Observations of C. nebulosus placed in various concentrations of Pemex Gulf of Mexico spill, oil accomodated in water. Two fish placed in each 20 gallon aquaria at 1730 hours on 8/7/79.

Time	Tank# Conc.	OBSERVATIONS at 23C and 35 o/oo					
		1* 100%	2* 50%	3* 25%	4 12.5%	5 5%	6 1%
1750 (8/7)		head shaking convulsing	alive poor vis.	equilibrium loss, head shaking	normal	normal	normal
1830 (8/7)		Coughing	Coughing irregular ventilation	Coughing irregular ventilation	normal	normal	normal
2010 (8/7)		Coughing irreg.-labored ventilation	Coughing irregular ventilation	Coughing irregular ventilation	normal	labored ventilation comp. to 1%	normal very calm
2130 (8/7)		As above	increased coughing irreg. vent.	As above	normal	one fish moving some, seems stress	As above
2230 (8/7)		As above	As above	as above	As above	Large fish swimming unnaturally	As above
0035 (8/8)		As above	as above	as above	As above	As above	As above
0215 (8/8)		Irregular ventilation Coughing 2-3 times/min.	As above ventilation not forced	Labored Ventilation	Coughing Irregular Ventilation	Coughing Large fish Active/labored Vent.	As above

Appendix Table 1 (cont.)

Time	Tank# Conc.	OBSERVATIONS at 23C and 35 o/oo					
		1* 100%	2* 50%	3* 25%	4 12.5%	5 5%	6 1%
0425 (8/8)		As above Water Clearing	As Above Water Clearing	As Above	As above	As above	As above
0630 (8/8)		Fish Active Coughing Clearing Cont.	Fish Active Clearing	Fish Active Coughing Clearing Cont	Calm	Fish Active	Fish Active
0900 (8/8)		Most Active Mouths Agape Rapid Ventil.	Rapid ventil.	As above	Ventil. Slight. high, but calm	Calm & normal	Calm & normal
1030 (8/8)		Active Rapid Ventil.	Active Rapid Ventil.	Active Ventil Norm.	Calm Normal	As Above	As above
1300 (8/8)		As Above	As Above	As Above	As above	As above	As above
1400 (8/8)		As Above Coughing	As Above Coughing	Moderate Ventilation Coughing	Ventil. Above norm., Active	Slightly active	As above
1515** (8/8)		Ventilation and Activity highest ———		→ decreasing to ———		→	Calm

*Fins erect at higher concentration quite frequently; rarely at lower concentrations.
**These conditions persisted until fish were terminated on 8/11/79 at 1730 hours. 96 hours exposure.

Appendix Table 2

Observations of fingerling C. nebulosus placed in various concentrations of Pemex Gulf of Mexico Oil Spill material accomodated in water. Six fish placed in each concentration at 1615 hours on 8/14/79.

Time / Tank# / Conc.	Observations at 23C and 35 o/oo					
	1 100%	2 50%	3 25%	4 12.5%	5 5%	6 Control
2035 (8/14)	Ventilation Rapid Sluggish	Sluggish	Sluggish Some Coughing	Swimming in spurts, one dying, coughing, rapid vent.	Rapid Vent., Coughing, Sluggish	Alert and in good condition
2400 (8/14)	"	" One Dead	"	" One Dead	"	"
0300 (8/15)	"	" One Dead	"	"	"	"
0515 (8/15)	One dying, Ventilation Rapid and Labored	Coughing, Active Swimming	Coughing and Active	Coughing, Calmer than 1,2,3	Alert and Active	"
0830 (8/15)	One Dead "	"	"	"	Calm	Calm
1000 (8/15)	"	"	"	"	"	"
1100 (8/15)	"	"	"	"	"	"
1400 (8/15)	"	"	"	"	"	"
1600 (8/15)	"	"	"	"	"	"
2050 (8/15)	"	" Tank Clearing	" Tank Clearing	" Tank Clearing	"	"

Appendix Table 2 (cont.)

Time	Tank# / Conc.	Observations at 23C and 35 o/oo					
		1 100%	2 50%	3 25%	4 12.5%	5 5%	6 Control
0800 (8/16)		"	"	" One Dead	" One Dead	" Two Dead	"
0930 (8/16)		Two Near Surface Bad Shape	"	"	"	"	"
1115 (8/16)		One near Surface One Dead Others "	"	"	"	"	"
1300 (8/16)		One Dead	"	"	"	"	"
1400 (8/16)		"	"	"	"	"	"
1500 (8/16)		One small fish looks bad, rest "	"	"	"	"	"
2100 (8/16)		"	"	"	"	"	One dead Rest Healthy
0815 (8/17)		" Rapid Vent.	" Rapid Vent.	" Two Dead	"	"	"
0930 (8/17)		" Swimming hard	" Rapid vent.	"	"	"	"
1100 (8/17)		"	"	"	"	"	"
1300 (8/17)		"	"	"	"	"	"

Appendix Table 2 (cont.)

Time	Tank# Conc.	Observations at 23C and 35 o/oo					
		1 100%	2 50%	3 25%	4 12.5%	5 5%	6 Control
1600 (8/17)		" One Dead	"	"	"	"	"
0800 (8/18)		"	"	"	"	" One Dead	"
1030 (8/18)		"	"	"	"	"	"
1615 (8/18) TERMINATION		One Dead One Live fish remains, sluggish, mod. tail rot.	Five fish alive all very sluggish	Three Alive, Healthy and Active.	Three Alive, Two healthy, one small - sluggish	Three Alive and healthy	Five fish in excellent shape
TOTAL FATALITIES		5	1*	3	3	3	1

* This tank cleared very fast and did not exhibit a toxicity effect in line with the other concentrations. It was noticed that this system had a very high rate of aeration compared to the other tanks, which may have resulted in a much faster breakdown of toxic components of the oil mixture.

Appendix Table 2a

Weight, standard and total lengths for subadult C. nebulosus utilized in toxic concentration studies on Mexican oil spill materials.

Conc.		Weight (g)	Std. Length (Cm)	Total Length (cm)	Avg. Wt. (g)
Fish Killed					
100%	1)	8.5	8.8	10.9	
	2)	4.5	6.8	8.6	
	3)	7.3	8.2	9.7	8.8
	4)	3.0	6.0	7.6	
	5)	20.6	11.4	13.8	
50%	1)	4.2	6.7	8.4	4.2
25%	1)	4.3	6.5	7.5	
	2)	6.2	7.1	8.7	5.4
	3)	5.8	7.0	8.5	
12.5%	1)	5.4	6.9	8.3	
	2)	5.0	6.7	8.1	4.1
	3)	2.0	5.3	6.5	
5%	1)	6.9	7.2	8.6	
	2)	25.9	12.2	15.0	13.4
	3)	7.3	7.7	9.3	
0% (Control)	1)	6.7	7.7	9.4	6.7
Fish Alive					
100%	1)	40.1	14.8	17.8	40.1
50%	1)	30.7	12.7	15.5	
	2)	19.1	11.2	13.7	
	3)	8.9	8.6	10.6	14.8
	4)	9.8	8.7	10.5	
	5)	5.6	7.4	9.1	
25%	1)	34.7	13.0	15.8	
	2)	26.9	12.4	15.0	25.9
	3)	16.0	10.3	12.4	
12.5%	1)	8.4	8.4	10.3	
	2)	6.1	7.7	9.5	9.5
	3)	14.0	9.8	12.2	
5%	1)	15.0	10.0	12.5	
	2)	10.0	8.4	10.4	10.1
	3)	5.2	6.9	8.8	
0% (Control)	1)	22.1	11.4	14.0	
	2)	14.6	10.4	12.7	
	3)	11.0	9.2	11.5	12.0
	4)	7.6	8.1	10.1	
	5)	4.9	6.9	8.6	

Appendix Note

Blazka Acclimation Notes

1. Nine fish exposed to 5% Ixtoc I oil accommodated in water. This 5% "mixture" was prepared by blending "mousse" with water to obtain a concentrated sample which was then diluted with two parts seawater to obtain what we will refer to as a 100% sample. Analysis of the content of these samples are underway at this time.
2. Initial exposure at 1630 hours on 8/13/79 in the Blazka exercise tank. Temperature 28C; Salinity 35 o/oo.
3. On 8/15/79 at 0830 hours four fish were dead. Three fish swimming on surface rapid ventilation, mouths agape, sluggish.
4. 8/15/79 at 1500 hours - Two more fish dead.
5. At 0800 on 8/16, final two fish dead.
6. Data on fish:

Weight (g)	T.L. (cm)	S.L. (cm)
178.0	28.0	24.5
90.6	23.8	20.7
178.2	28.4	25.8
166.4	27.8	24.5
142.6	26.9	23.5
109.8	24.0	21.5
100.2	23.3	20.0
133.2	25.7	22.2
109.2	24.6	20.6

Notes:

1. All fish exhibited light to moderate tail rot. Oil and lesions on gills.
2. Total exposure time = 63.5 hours.
3. Static tests at 23°C - Blazka acclimation at 28°C 5C difference + swimming stress produced by current apparently were too severe.